PB83-153809

Design of 301 (h) Monitoring Programs for Municipal Wastewater Discharges to Marine Waters

Tetra Tech., Inc.
Bellevue, WA

Prepared for

Environmental Protection Agency
Washington, DC

Nov 82

U.S. Department of Commerce
National Technical Information Service
NTIS

PB83-153809

United States
Environmental Protection
Agency

Office of Water
Program Operations (WH 546)
Washington DC 20460

November 1982
430/9-82-010

EPA

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U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

BIBLIOGRAPHIC INFORMATION

PB83-153809

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Nov 82

PERFORMER: Tetra Tech, Inc., Bellevue, WA.
Contract EPA-68-01-5906

SPONSOR: Environmental Protection Agency, Washington, DC.
Office of Water Program Operations.
EPA-430/982-010

See also PB83-153817.

Under Section 301(h) of the Clean Water Act of 1977 as amended by the Municipal Wastewater Treatment Construction Grant Amendments of 1981, publicly owned treatment works (POTWs) may apply for a variance from the secondary treatment requirements for discharge into marine waters. The guidance provided in this document has been developed to help meet the general monitoring requirements of the 301(h) program. This document was prepared in order to provide guidance for designing monitoring programs that will meet regulatory requirements and allow continuing assessment of the impact of less-than-secondary discharges on the receiving water marine environment.

KEYWORDS: *Municipalities, *Sewage disposal, *Oceans, *Monitoring.

Available from the National Technical Information Service, Springfield, Va. 22161

PRICE CODE: PC A07/MF A01

DESIGN OF 301(h) MONITORING PROGRAMS FOR MUNICIPAL WASTEWATER DISCHARGES TO MARINE WATERS

November, 1982

by

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Contract Number 68-01-5906

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EPA REVIEW NOTICE

This report was prepared under the direction of the Office of Marine Discharge Evaluation (WH-546), Office of Water Program Operations, Office of Water, U.S. Environmental Protection Agency, 401 M Street, S.W., Washington D.C., 20460, (202) 755-9231.

This report has been reviewed by the Office of Water and the Office of Research and Development, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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CHAPTER I

OVERVIEW OF MONITORING REQUIREMENTS

INTRODUCTION

Under Section 301(h) of the Clean Water Act of 1977 as amended by the Municipal Wastewater Treatment Construction Grant Amendments of 1981, publicly owned treatment works (POTWs) may apply for a variance from the secondary treatment requirements for discharge into marine waters. Each applicant is required to submit a detailed technical evaluation of the discharge and its effects on the marine environment to demonstrate compliance with the seven statutory criteria listed under Section 301(h). If a variance were granted, monitoring would be required [Section 301(h)(3)] to assess the impact of the modified discharge on marine biota. EPA regulations implementing Section 301(h) are set forth in 40 CFR Part 125, Subpart G, as amended in November, 1982.

The guidance provided in this document has been developed to help meet the general monitoring requirements of the 301(h) program. References to applicable water quality standards and requirements are not intended to replace specific state requirements. Applicants must also check with the appropriate state and local agencies for any specific monitoring requirements applicable to their circumstances.

This document was prepared in order to provide guidance for designing monitoring programs that will meet regulatory requirements (40 CFR 125.62) and allow continuing assessment of the impact of less-than-secondary discharges on the receiving water marine environment. It provides supplemental guidance on designing monitoring programs to that included in the Revised Section 301(h) Technical Support Document (Tetra Tech 1982) which is available by writing to the Office of Marine Discharge Evaluation (WH-546), U.S. Environmental Protection Agency, 401 M Street, S.W., Washington, D.C., 20460. The guidance provided in these documents is advisory only; its use is not required. However, EPA believes that Section 301(h) applicants will benefit substantially by following the guidance and procedures provided in these documents.

The amended 301(h) regulations include a number of changes to the monitoring program requirements contained in the original 301(h) regulations promulgated in 1979. While the basic objectives of the overall monitoring requirements remain the same, many of the original detailed requirements were deleted from the amended regulations so that each applicant will have the flexibility to design a cost-effective monitoring program to meet its individual circumstances. This is especially true for small applicants that discharge into depths greater than 10 meters with negligible seabed accumulation of suspended solids [40 CFR 125.62(b)(2)].

Much of the guidance in this document is directed towards large dischargers. It covers a wide range of possibilities that might be encountered when developing 301(h) monitoring programs, including complex waste streams and discharges into sensitive ecosystems.

Users of this guidance document should keep in mind that the level of effort for each 301(h) monitoring program must be keyed to the individual circumstances of each discharge and corresponding receiving water situation. A monitoring program will not have to be as extensive for smaller dischargers as for large dischargers. A monitoring program for a waste discharge comprised primarily of domestic wastes does not have to be as comprehensive as a program to monitor the impact of a discharge with large amounts of industrial and/or toxic wastes. The frequency of sampling required for a resilient, high energy, or otherwise nonsensitive receiving water environment will be considerably less than for a sensitive ecosystem. A minimally acceptable monitoring program, then, will be based on a balance of several factors, including the size of the discharge, the character of the waste, and the sensitivity and variability of the receiving water environment. In addition, a test of monitoring program practicability should include consideration of the technical feasibility of available measurement procedures during a variety of weather and sea conditions.

Those EPA tentative decision documents which recommend Section 301(h) variances will highlight site-specific items which must be addressed in the applicant's proposed monitoring program. The 301(h) decision document should, therefore, be analyzed carefully and the monitoring requirements therein reflected in the design of the applicant's final monitoring program. The Technical Evaluation or Technical Review Reports on individual 301(h) applications should also be used as a reference in the design of final monitoring program proposals.

OBJECTIVES OF MONITORING

Monitoring programs under 40 CFR 125.62 for dischargers receiving modified NPDES permits under Section 301(h) of the Clean Water Act should be designed to:

- Document short- and long-term effects of the discharge on receiving water, sediments, and biota; also, on beneficial uses of the receiving water
- Determine compliance with NPDES permit terms and conditions
- Assess the effectiveness of toxic control programs.

While divided into general biological, water quality, and effluent monitoring components, in general, the monitoring program should focus upon demonstrating the discharge's compliance with applicable standards and permit conditions, and demonstrating predictable relationships between discharge characteristics and impacts upon the marine receiving water quality and the marine biota. Although each general monitoring component may involve sampling at different locations for different variables and at different times, it should not be considered as a separate and individual activity, but as an integrated study. In this manner, the permittee should be able to gain the most meaningful data on an assessment of the impacts of the discharge. Further, once an adequate background data base is established and predictable relationships among the biological, water quality, and effluent monitoring variables are demonstrated, it should be possible for many 301(h) permittees, especially those with small discharges, to scale down the intensity of certain elements of their field monitoring studies.

Applicants may wish to expand their monitoring programs beyond the minimum required to further demonstrate the impact or lack of impact of their discharge on the environment. In addition, they may wish to exploit unique opportunities provided by 301(h) to add to the body of knowledge on effects of marine discharges on the receiving water environment and marine ecosystems. The potential benefits to the municipality would be in assessing long-range wastewater treatment and disposal needs and alternatives. Additionally, applicants discharging in the same geographic proximity may wish to develop an areawide assessment of marine discharge environmental impacts. Applicants should consider, also, that the monitoring data provided will be used by EPA to assess whether 301(h) variances should be renewed following expiration of the initial variances/permits.

301(h) Requirements

The monitoring requirements specified by 40 CFR Part 125.62 provide for monitoring programs comprised of three elements: (1) biological monitoring, (2) water quality monitoring, and (3) effluent monitoring. In addition, applicants must demonstrate in their monitoring program proposals that they possess the economic, personnel, technical, and other resources necessary to implement their proposed programs. The biological and water quality sampling must be able to detect variations over time and space as those changes relate to the permittee's discharge. Monitoring must be conducted at the current discharge site before and after any improvements are implemented and at the site of new or relocated discharges. Sampling times should include critical environmental periods and both typical and unusual meteorological or oceanographic conditions. Biological programs for large permittees and some small permittees must include field surveys of affected or potentially affected biota, bioaccumulation studies, and an assessment of the condition and productivity of commercial and recreational fisheries. Water quality samples must be from stations selected to assess compliance with water quality standards in the vicinity of the zone of initial dilution (ZID), and beyond the ZID. The toxics monitoring program must determine the effectiveness of industrial pretreatment and nonindustrial toxics control programs. An adequate toxics monitoring program will also aid the implementation of toxics control programs and the biological monitoring efforts.

The applicants must provide EPA with sufficient information on quality assurance[a] and control procedures to document compliance with accepted scientific practice. The monitoring plan, therefore, should discuss quality assurance in general, and specific data generation sections of the plan should reflect individual details of quality control.

State Requirements

The monitoring program must document compliance with all applicable water quality standards. Some states have specific monitoring requirements and/or recommendations on parameters to be sampled, station locations, sampling frequencies, and analytical methods. Table 1, for example, shows the parameters recommended by the California Ocean Plan guidelines (CSWRCB 1972 and 1978). The parameters included in most State standards for receiving waters are dissolved oxygen, pH, coliform bacteria, and suspended solids or a surrogate. The standards have been expressed as a maximum allowable pollutant concentration, a maximum allowable deviation from background concentrations, a statistically significant difference between stations, or as a prescription against harm to biota or degradation of beneficial uses of the water body.

NPDES Requirements

An NPDES permit is required, by Section 402 of the Clean Water Act, for all discharges of pollutants to navigable waters. The permits specify effluent limitations plus effluent sample types (e.g., grab or 24-hour composite) and sampling frequencies for assessing compliance. In some cases the permits include receiving water monitoring requirements. The NPDES requirements should be used by 301(h) applicants as a basis of decision making on influent and effluent monitoring. NPDES permit sampling

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[a] EPA policy initiated by the Administrator in a memorandum, dated May 30, 1979, stipulates that all environmental monitoring and measurement efforts mandated or supported by EPA must have quality assurance project plans (see Chapter V, Quality Control).

TABLE 1. MONITORING REQUIRED BY CALIFORNIA OCEAN PLAN

Parameter	Location of Monitoring Water Supply	 Untreated Wastewater	 Effluent	 Receiving Water	 Sediments
Flow		X	X		
Bacteriological			X	X	
Grease and Oil		X	X	X	
Floating Particulates			X	X	
Suspended Solids		X	X	X	
Settleable Solids			X		
Turbidity			X		
pH	X	X	X	X	
Arsenic	X	X	X		X
Cadmium	X	X	X		X
Total Chromium	X	X	X		X
Copper	X	X	X		X
Lead	X	X	X		X
Mercury	X	X	X		X
Nickel	X	X	X		X
Silver	X	X	X		X
Zinc	X	X	X		X
Cyanide		X	X		X
Phenolic Compounds	X	X	X		X
Total Chlorine Residual			X		
Ammonia Nitrogen				X	
Total Identifiable Chlorinated Hydrocarbon	X	X	X		X
Toxicity		X	X		
Radioactivity	X	X	X		X
Salinity				X	
Temperature			X	X	
Biochemical Oxygen Demand		X	X		X
Total Phosphate	X	X	X		
Total Nitrogen	X	X	X		
Dissolved Oxygen				X	
Discoloration				X	
Light Transmittance				X	
Fish and Macroinvertebrates				X	
Sediment Sulphides					X
Particle Size Distribution					X
Benthic Biota					X

NOTE: X means monitoring required.

Source: California State Water Resources Control Board (1972, 1978).

specifications will be supplemented (usually more frequent monitoring or addition of parameters) to meet 301(h) objectives and/or state requirements. Additional requirements to meet 301(h) objectives will consider 301(h) related effluent limitations, plant flow characteristics, initial dilution ratios, receiving water characteristics, and biological communities and beneficial uses to be protected.

Other Requirements

For each discharge, an investigation should determine if there are other water quality standards applicable to the given water body or other monitoring program requirements. For example, the Interstate Sanitation Commission has requirements relating to wastewater discharges in the New York Harbor area. In California, basin plans developed by regional boards or agencies contain requirements in addition to those found in the California Ocean Plan.

CHAPTER II

TREATMENT PLANT AND EFFLUENT MONITORING

OBJECTIVES

Plant monitoring (influent and effluent) is primarily required to determine compliance with NPDES permit conditions and water quality standards. In addition, influent and effluent monitoring provides indicators for assessment of treatment plant performance. High effluent pollutant concentrations may be due to plant malfunctions or overloads; thus, plant monitoring can be used to identify problems and improve performance. Effluent monitoring also provides information on waste characteristics and flows for use in interpreting water quality and biological data.

Monitoring programs for toxic substances and pesticides are required as part of the 301(h) regulations and should be designed to:

- Determine the potential for toxicity to aquatic life and risk to human health from toxic chemical substances discharged to marine waters, and to

- Evaluate the effectiveness of industrial source control pretreatment programs and nonindustrial toxic control programs.

The first objective can be attained by measuring toxic chemical substances in the effluent and in selected samples taken from the receiving water sediments and organisms used in biomonitoring protocols. The second objective can be attained by measuring toxic substances in the treatment plant influent and comparing trends. Sources of toxicants, whether industrial or nonindustrial, are analyzed and identified as part of the toxics control program.

SPECIFICATIONS FOR TREATMENT PLANT AND EFFLUENT MONITORING

Major considerations in the design of plant sampling programs include the specification of: sampling locations, parameters to be measured, collection and analytical methods, and sampling frequencies.

Sampling Locations

Specific locations at the treatment plant for influent and effluent sampling may be included in the NPDES permit. In the 301(h) monitoring program only influent and effluent sampling points are specified, although sampling at intermediate points within the plant may be useful for monitoring individual treatment unit performance. Sampling at various points in the collection system may also be necessary to isolate sources of toxic substances.

For conventional pollutants and nutrients, influent samples should generally be collected just downstream of the coarse screens or grit chamber. If multiple waste streams enter the plant and a representative sample cannot be collected, a flow-composite sample may be used for influent analysis. Effluent samples should be collected downstream of any chlorination or disinfection units. Samples should be taken as close to the start of the outfall as possible. An example of such a sampling point would be the effluent pumping station. Separate samples should be taken if two outfalls are used and the effluent which enters the outfalls comes from different parts of the treatment plant. When emergency bypasses are made to a different outfall or discharge point, due to high inflows or treatment plant problems, separate samples of the bypassed flows should be taken.

Sampling for toxic pollutants should include hourly grab samples collected over a 24-hour period and composited in proportion to the flow. Influent samples should be taken upstream of the plant intake works (prior to the grit chamber, if possible) and the total (unfiltered) sample should be analyzed. Effluents should be sampled after treatment and just prior to entering the outfall pipe. If the effluent is chlorinated, samples should be taken upstream and downstream of the chlorination unit.

Parameters

The treatment plant monitoring parameters required by an NPDES permit for a typical large discharger include conventional pollutants, nutrients, and toxicants. Influent monitoring normally includes volumetric flow rate, BOD_5, suspended solids, pH, and grease and oil. The suspended solids and BOD_5 measurements are used to determine removal efficiencies and to detect changes in the character of the waste stream. Measurements of pH and grease and oil are used to determine the need for and success of any pretreatment programs. Other influent monitoring parameters which may be required are total phosphorus, total nitrogen or specific forms of nitrogen, settleable solids, COD, and temperature. These variables may be needed to further characterize the influent and to monitor treatment plant performance.

The parameters to be measured in the effluent include requirements of the water quality standards, NPDES permit, and any additional variables needed to interpret water quality and biological data. Plant effluent monitoring should normally include volumetric flow rate, dissolved oxygen, BOD_5, suspended solids, settleable solids, temperature, total and fecal coliform bacteria, grease and oil, and pH. If the effluent is chlorinated, total chlorine residual is typically monitored. To aid in evaluating the importance of chlorination in forming persistent, possibly hazardous, chloro-organics, a careful record of the total mass of chlorine used per unit of flow should be reported. If other forms of disinfection are used, type and dosage should be reported. Other variables which may be required include floating particulates, total phosphorus, total nitrogen, ammonia or other forms of nitrogen, and COD.

In addition to the above parameters, Section 125.62(d) of the amended 301(h) regulations requires each applicant, to the extent practicable, to monitor toxic substances and pesticides [see 40 CFR 125.8(u) and (m)] in the effluent. A list of the 129 toxic pollutants is provided later in Table 3.

Sample Collection and Frequency

The type of sampling equipment to be used and sampling frequencies depend on the size and nature of the discharge. Sampling frequency and type of sample should be determined based on the variability of the influent and effluent characteristics and the flow so that the data collected will be representative of the discharge. In general, volumetric flow rates of the influent and effluent should be measured continuously using automatic equipment. Hourly and average daily flow rates should be recorded. Daily effluent and influent samples for BOD and suspended solids should be taken. Twenty-four hour flow-composite samples are recommended. Nutrient sampling may be done weekly or monthly using grab samples selected randomly or 24-hour flow-composite samples collected on randomly selected days during the sampling period. Measurements of effluent pH should be done on daily grab samples taken at different times each day. Daily grab samples are typically taken for total and fecal coliform bacteria.

Generally, a randomly selected date within a defined sampling period, coordinated with other sampling (i.e., for conventional pollutants) during wet and dry flow periods, should be chosen for influent and effluent sampling for toxic pollutants. Flows caused by bypass events at the POTWs should also be considered for sampling and analysis.

The sampling frequency for toxic pollutants depends on such factors as the size and location of the discharge, the types and quantities of toxic pollutants present, and the sensitivity and beneficial uses of the receiving water marine environment. More frequent sampling should occur for POTWs with large discharges, significant types and quantities of toxic pollutants, and sensitive receiving waters. All large POTWs and those small POTWs that cannot certify they have no known or suspected sources of toxic pollutants or pesticides need to establish baseline analyses for toxic pollutants and pesticides present in their current discharge (40 CFR Part 125.64). Toxic substance monitoring is required of all 301(h) waiver recipients to help verify the type and quantity of the compounds identified in the discharger's 301(h) application and to determine if significant changes occur over time.

It is recommended that at least annual representative wet and dry weather 24-hour composite sampling and analyses be undertaken. More frequent sampling and analyses may be required depending on the type of substances found in the wastewater and discharge or the sensitivity of the receiving waters.

More frequent plant monitoring may be necessary for both conventional and toxic pollutants during the first year of the program to obtain reliable estimates of when maximum waste load periods occur and the magnitude of peak concentrations. In some cases the relationship between concentrations of a variable (e.g., BOD or suspended solids) and volumetric flow rates is not well known. Sampling at times of minimum, average, and maximum hourly flow rates on a monthly basis for the first year should help define concentration-flow rate relationships and allow better interpretation of the receiving water quality data.

Analytical Methods for Toxic Substances

Regulations have been proposed on allowable holding times and analytical procedures for toxic substances [45 Fed. Reg. No. 231 p. 79318-79379 (November 28, 1980)]. The final regulations establishing test procedures for the analysis of toxic substances have not yet been published. Recommended holding times, container requirements, and preservation methods are listed in Table 2. Recommended analytical methods are shown in Table 3 for the priority pollutants. Analytical methods for the six pesticides (methoxychlor, mirex, guthion, malathion, parathion, and demeton) can be found in Watts (1980) and U.S. EPA (1978).

For many dischargers it will be necessary to contract with outside laboratories for the analytical work. The laboratories selected should be state certified according to U.S. EPA approved procedures. Sampling, holding and analysis procedures, and equipment should comply with state and federally approved methods.

Toxic Substance Data Reporting

Quality assurance information should be transferred quarterly to EPA and should include copies of quality control charts used in the laboratory and the results of replicate, split, spiked, and blank sample analyses. The laboratory results submitted should include the calibration standards used, copies of the calibration curves used, and the frequency of calibration runs.

TABLE 2. CONTAINERS, PRESERVATION, AND HOLDING TIMES FOR SELECTED GROUPS OF TOXIC CHEMICALS

Parameter	Container[a]	Preservation[b]	Maximum Holding Time[c]
Metals			
Chromium VI	P,G	Cool, 4°C	24 hours
Mercury	P,G	HNO_3 to pH > 2 0.05% $K_2Cr_2O_7$	28 days
Metals in Table 3 (except above)	P,G	HNO_3 to pH > 2	6 months
Asbestos	P	1 ml 2.71% $HgCl_2$	5 days
Cyanide (total and amenable to chlorination)	P,G	Cool, 4°C NaOH to pH > 12 0.008% $Na_2S_2O_3$[e]	14 days
Organic Compounds[d]			
Extractable (including phthalates, nitrosamines, organochlorine pesticides, PCBs, nitroaromatics, isophorone, polynuclear aromatic hydrocarbons, haloethers, chlorinated hydrocarbons and TCDD)	G, teflon-lined cap	Cool, 4°C 0.008% $Na_2S_2O_3$[e]	7 days (until extraction) 30 days (after extraction)
Extractables (phenols)	G, teflon-lined cap	Cool, 4°C H_2SO_4 to pH > 2 0.008% $Na_2S_2O_3$[e]	7 days (until extraction) 30 days (after extraction)
Purgeables (halocarbons and and aromatics)	G, teflon-lined septum	Cool, 4°C 0.008% $Na_2S_2O_3$[e]	14 days

TABLE 2. (Continued)

Parameter	Container[a]	Preservation[b]	Maximum Holding Time[c]
Purgeables (acrolein and acrylonitrile)	G, teflon-lined septum	Cool, 4°C 0.008% $Na_2S_2O_3$[e]	3 days
Pesticides	G, teflon-lined cap	Cool, 4°C 0.008% $Na_2S_2O_3$[e]	7 days (until extraction) 30 days (after extraction)
Phenols	P,G	Cool, 4°C H_2SO_4 to pH > 2	28 days

[a] Polyethylene (P) or Glass (G).

[b] Sample preservation should be performed immediately upon sample collection. For composite samples each aliquot should be preserved at the time of collection. When use of an automatic sampler makes it impossible to preserve each aliquot, then samples may be preserved by maintaining at 4°C until compositing and sample splitting is completed.

[c] Samples should be analyzed as soon as possible after collection. The times listed are the maximum times that samples may be held before analysis and still considered valid. Samples may be held for longer periods only if the permittee, or monitoring laboratory has data on file to show that the specific types of samples under study are stable for the longer time.

Some samples may not be stable for the maximum time period given in the table. A permittee or monitoring laboratory is obligated to hold the sample for a shorter time if knowledge exists to show this is necessary to maintain sample stability.

[d] Guidance applies to samples to be analyzed by GC, HPLC, or GC/MS for specific organic compounds.

[e] Should only be used in the presence of residual chlorine.

NOTE: If preservative is unavailable for organic compounds, recommended holding time is 48 hours at 4°C.

Source: U.S. Environmental Protection Agency (1979a).

TABLE 3. LIST OF APPROVED ANALYTICAL METHODS FOR SELECTED TOXIC CHEMICALS (PRIORITY POLLUTANTS)

Parameter and Units	Methods (EPA Method Number)
1. Acenaphthene, ug/l	GC or HPLC (610), GC/MS (625)
2. Acrolein, ug/l	GC or HPLC (603), GC/MS (624)
3. Acrylonitrile, ug/l	GC or HPLC (603), GC/MS (624)
4. Benzene, ug/l	GC (602), GC/MS (624)
5. Benzidine, ug/l	HPLC (605), Oxidation-colormetric, GC/MS (625)
6. Carbon tetrachloride (tetrachloromethane), ug/l	GC (601), GC/MS (624)
Chlorinated Benzenes (other than dichlorobenzenes)	
7. Chlorobenzene, ug/l	GC (601), (602), GC/MS (624)
8. 1,2,4-trichlorobenzene, ug/l	GC (612), GC/MS (625)
9. Hexachlorobenzene, ug/l	GC (612), GC/MS (625)
Chlorinated Ethanes	
10. 1,2-dichloroethane, ug/l	GC (601), GC/MS (624)
11. 1,1,1, trichloroethane ug/l	GC (601), GC/MS (624)
12. Hexachloroethane, ug/l	GC (612), GC/MS (625)
13. 1,1-dichloroethane, ug/l	GC (601), GC/MS (624)
14. 1,1,2-trichloroethane, ug/l	GC (601), GC/MS (624)
15. 1,1,2,2-tetrachloroethane, ug/l	GC (601), GC/MS (624)
16. Chloroethane, ug/l	GC (601), GC/MS (624)
Chloroalkylethers (chloromethyl, chloroethyl and mixed ethers)	
17. Bis (chloromethyl) ether[a]	
18. Bis (2-chloroethyl) ether, ug/l	GC (611), GC/MS (625)
19. 2-chloroethyl vinyl ether (mixed),ug/l	GC (601), GC/MS (624)
Chlorinated Naphthalene	
20. 2-chloronaphthalene	GC (612), GC/MS (625)

TABLE 3. (Continued)

Chlorinated Phenols (other than those listed elsewhere, includes trichlorophenols and chlorinated cresols)	
21. 2,4,6-trichlorophenol, ug/l	GC (604), GC/MS (625)
22. Para-chloro meta-cresol, ug/l	GC (604), GC/MS (625)
23. Chloroform (trichloromethane), ug/l	GC (601), GC/MS (624)
24. 2-chlorophenol, ug/l	GC (604), GC/MS (625)
Dichlorobenzenes	
25. 1,2-dichlorobenzene, ug/l	GC (601, 602, 612), GC/MS (625)
26. 1,3-dichlorobenzene, ug/l	GC (601, 602, 612), GC/MS (625)
27. 1,4-dichlorobenzene, ug/l	GC (601, 602, 612), GC/MS (625)
Dichlorobenzidine	
28. 3,3-dichlorobenzidine, ug/l	HPLC (605), GC/MS (625)
Dichloroethylenes	
29. 1,1-dichloroethylene, ug/l	GC (601), GC/MS (624)
30. 1,2-trans-dichloroethylene, ug/l	GC (601), GC/MS (624)
31. 2,4-dichlorophenol, ug/l	GC (604), GC/MS (625)
Dichloropropane and Dichloropropene	
32. 1,2-dichloropropane, ug/l	GC (601), GC/MS (624)
33. 1,2-dichloropropylene (1,2-dichloropropene), ug/l	GC (601), GC/MS (624)
34. 2,4-dimethylphenol	GC (604), GC/MS (625)
Dinitrotoluenes	
35. 2,4-dinitrotoluene, ug/l	GC (609), GC/MS (625)
36. 2,6-dinitrotoluene, ug/l	GC (609), GC/MS (625)
37. 1,2-diphenylhydrazine, ug/l	GC/MS (625)
38. Ethylbenzene, ug/l	GC (602), GC/MS (624)
39. Fluoranthene, ug/l	GC or HPLC (610), GC/MS (625)

TABLE 3. (Continued)

	Haloethers (other than those listed elsewhere)	
40.	4-chlorophenyl phenyl ether, ug/l	GC (611), GC/MS (625)
41.	4-bromophenyl phenyl ether, ug/l	GC (611), GC/MS (625)
42.	Bis (2-chlorisopropyl) ether, ug/l	GC (611), GC/MS (625)
43.	Bis (2-chloroethoxy) methane, ug/l	GC (611), GC/MS (625)
	Halomethanes (other than those listed elsewhere)	
44.	Methylene chloride (dichloromethane), ug/l	GC (601), GC/MS (624)
45.	Methyl chloride (chloromethane), ug/l	GC (601), GC/MS (624)
46.	Methyl bromide (bromomethane), ug/l	GC (601), GC/MS (624)
47.	Bromoform (tribromomethane), ug/l	GC (601), GC/MS (624)
48.	Dichlorobromomethane, μg/l	GC (601), GC/MS (624)
49.	Trichlorofluoromethane[b]	
50.	Dichlorodifluoromethane[b]	
51.	Chlorodibromomethane, ug/l	GC (601), GC/MS (624)
52.	Hexachlorobutadiene, ug/l	GC (612), GC/MS (625)
53.	Hexachlorocyclopentadiene, ug/l	GC (612), GC/MS (625)
54.	Isophorone, ug/l	GC (609), GC/MS (625)
55.	Naphthalene, ug/l	GC or HPLC (610), GC/MS (625)
56.	Nitrobenzene, ug/l	GC (609), GC/MS (625)
	Nitrophenols	
57.	2-nitrophenol, ug/l	GC (604), GC/MS (625)
58.	4-nitrophenol, ug/l	GC (604), GC/MS (625)
59.	2,4-dinitrophenol, ug/l	GC (604), GC/MS (625)
60.	4,6-dinitro-o-cresol, ug/l	GC (604), GC/MS (625)
	Nitrosamines	
61.	N-nitrosodimethylamine, ug/l	GC (607), GC/MS (625)
62.	N-nitrosodiphenylamine, ug/l	GC (607), GC/MS (625)
63.	N-nitrosodi-n-propylamine, ug/l	GC (607), GC/MS (625)
64.	Pentachlorophenol, ug/l	GC (604), GC/MS (625)
65.	Phenol, ug/l	GC (604), GC/MS (625)

TABLE 3. (Continued)

	Phthalate Esters	
66.	Bis (2-ethylhexyl) phthalate, ug/l	GC (606), GC/MS (625)
67.	Butyl benzyl phthalate, ug/l	GC (606), GC/MS (625)
68.	Di-n-butyl phthalate, ug/l	GC (606), GC/MS (625)
69.	Di-n-octyl phthalate, ug/l	GC (606), GC/MS (625)
70.	Diethyl phthalate, ug/l	GC (606), GC/MS (625)
71.	Dimethyl phthalate, ug/l	GC (606), GC/MS (625)
	Polynuclear Aromatic Hydrocarbons	
72.	Benzo (a) anthracene (1,2-benzyanthracene), ug/l	GC or HPLC (610), GC/MS (625)
73.	Benzo (a) pyrene (3,4-benzopyrene), ug/l	GC or HPLC (610), GC/MS (625)
74.	3,4-benzofluoranthene, ug/l	GC or HPLC (610), GC/MS (625)
75.	Benzo (k) fluoranthene (11,12-benzofluoranthene), ug/l	GC or HPLC (610), GC/MS (625)
76.	Chrysene, ug/l	GC or HPLC (610), GC/MS (625)
77.	Acenaphthylene, ug/l	GC or HPLC (610), GC/MS (625)
78.	Anthracene, ug/l	GC or HPLC (610), GC/MS (625)
79.	Benzo (ghi) perylene (1,12 benzoperylene), ug/l	GC or HPLC (610), GC/MS (625)
80.	Fluorene, ug/l	GC or HPLC (610), GC/MS (625)
81.	Phenanthrene, ug/l	GC or HPLC (610), GC/MS (625)
82.	Dibenzo (a,h) anthracene (1,2,5,6-dibenzanthracene), ug/l	GC or HPLC (610), GC/MS (625)
83.	Indeno (1,2,3-cd) pyrene, ug/l	GC or HPLC (610), GC/MS (625)
84.	Pyrene, ug/l	GC or HPLC (610), GC/MS (625)
85.	Tetrachloroethylene (tetrachloroethene), ug/l	GC (601), GC/MS (624)
86.	Toluene, ug/l	GC (602), GC/MS (624)
87.	Trichloroethylene (trichloroethene), ug/l	GC (601), GC/MS (624)
88.	Vinyl chloride (chloroethylene), ug/l	GC (601), GC/MS (624)
	Pesticides and Metabolites	
89.	Aldrin, ug/l	GC (608), GC/MS (625)
90.	Dieldrin, ug/l	GC (608), GC/MS (625)
91.	Chlordane (technical mixture and metabolites), ug/l	GC (608), GC/MS (625)

TABLE 3. (Continued)

DDT and Metabolites	
92. 4,4'-DDT, ug/l	GC (608), GC/MS (625)
93. 4,4'-DDE (p,p-DDX), ug/l	GC (608), GC/MS (625)
94. 4,4'-DDD (p,p-TDE), ug/l	GC (608), GC/MS (625)
Endosulfan and Metabolities	
95. α-endosulfan-Alpha, ug/l	GC (608), GC/MS (625)
96. β-endosulfan-Beta, ug/l	GC (608), GC/MS (625)
97. Endosulfan sulfate, ug/l	GC (608), GC/MS (625)
Endrin and Metabolites	
98. Endrin, ug/l	GC (608), GC/MS (625)
99. Endrin aldehyde, ug/l	GC (608), GC/MS (625)
Heptachlor and Metabolites	
100. Heptachlor, ug/l	GC (608), GC/MS (625)
101. Heptachlor epoxide, ug/l	GC (608), GC/MS (625)
Hexachlorocyclohexane (all isomers)	
102. α-BHC-Alpha, ug/l	GC (608), GC/MS (625)
103. β-BHC-Beta, ug/l	GC (608), GC/MS (625)
104. γ-BHC (lindane) -Gamma, ug/l	GC (608), GC/MS (625)
105. δ-BHC-Delta, ug/l	GC (608), GC/MS (625)
Polychlorinated Biphenyls	
106. PCB-1242 (Aroclor 1242), ug/l	GC (608), GC/MS (625)
107. PCB-1254 (Aroclor 1254), ug/l	GC (608), GC/MS (625)
108. PCB-1221 (Aroclor 1221), ug/l	GC (608), GC/MS (625)
109. PCB-1232 (Aroclor 1232), ug/l	GC (608), GC/MS (625)
110. PCB-1248 (Aroclor 1248), ug/l	GC (608), GC/MS (625)
111. PCB-1260 (Aroclor 1260), ug/l	GC (608), GC/MS (625)
112. PCB-1016 (Aroclor 1016), ug/l	GC (608), GC/MS (625)

TABLE 3. (Continued)

	Miscellaneous Substances (including metals, organic compounds not listed elsewhere, and asbestos)	
113.	Toxaphene, ug/l	GC (608), GC/MS (625)
114.	Antimony (total), ug/l	AA (204.2)
115.	Arsenic (total), ug/l	AA (206.2)
116.	Asbestos (fibrous), chrysotile fibers MFL (million fibers per liter)	TEM
117.	Beryllium (total), ug/l	AA (210.2)
118.	Cadmium (total), ug/l	AA (213.1)
119.	Chromium (total), ug/l	AA (218.1 or .2 or .3)
	(IV), ug/l	AA (218.4)
120.	Copper (total), ug/l	AA (220.2)
121.	Cyanide (total), ug/l	Titrimetric, Spectrophotometric (335.2)
122.	Lead (total), ug/l	AA (239.2)
123.	Mercury (total), ug/l	AA (245.1 or .2)
124.	Nickel (total), ug/l	AA (249.2)
125.	Selenium (total), ug/l	AA (270.2)
126.	Silver (total),ug/l	AA (272.2)
127.	Thallium (total), ug/l	AA (279.2)
128.	Zinc (total), ug/l	AA (289.2)
129.	2,3,7,8-tetrachlorodibenzo-p-dioxin (TCCD), ug/l	GC/MS (613), (625)

[a] Bis (chloromethyl) ether was removed from the toxic pollutant list (U.S. EPA 1981a).

[b] Dichlorodifluoromethane and trichlorofluoromethane were removed from the toxic pollutant list (U.S. EPA 1981b).

Note:
GC = Gas chrom tography.
HPLC = High performance liquid chromatography.
GC/MS = Gas chromatography coupled with mass spectrometry.

Source: U.S. EPA (1979a).

AA = Atomic absorption.

Source: U.S. EPA (1979b).

TEM = Transmission electron microscopy.

Source: Anderson and Long (1980).

The results of screening measurements for the priority pollutants in the effluent should be included in the monitoring reports. The results of the chemical analyses should, where possible, be reported as measured rather than less-than-certain values. If the analytical results were below the limit of detection, this should be noted on the data sheet and the value given as less than the actual limit of detection (e.g., < 10 ug/l). The list of compounds identified in previous screenings should be compared to the new results. The estimated concentration after initial dilution should be computed for each toxicant. These values after initial dilution should be compared to available criteria for marine waters [45 Fed. Reg. No. 231 pp. 79318-79379 (November 28, 1980)]. Those compounds which exceed the criteria should be added to the list of toxicants subject to bioassay. Toxicants found which can be bioaccumulated, but not previously included in the sediment monitoring program, should be added to that program.

CHAPTER III

RECEIVING WATER QUALITY AND SEDIMENT MONITORING

OBJECTIVES

To determine compliance with water quality standards and the 301(h) criteria, the receiving water quality monitoring program must document water quality in the vicinity of the Zone of Initial Dilution (ZID) boundary, at control or reference stations, and at areas beyond the ZID where discharge impacts might reasonably be expected. Monitoring must reflect conditions during all critical environmental periods as identified in the 301(h) applications. If currently available data are not adequate to predict when critical periods will occur, then greater monitoring effort may be necessary to demonstrate that water quality data are collected under the appropriately critical conditions. Examples of such critical conditions are periods of anadromous fish spawning runs, juvenile fish migrations or feedings, high wastewater loadings, high water temperature, and low flushing rate.

The applicant's historical sampling programs, together with new requirements associated with 301(h) permit conditions, will be the most useful guide for designing an adequate receiving water monitoring program. Changes in station locations, parameters, or frequencies may be required to rectify deficiencies in historical programs.

SPECIFICATIONS FOR WATER AND SEDIMENT MONITORING

Station Locations

Section 125.61 of the amended 301(h) regulations requires that water quality be maintained to assure the protection of public water supplies, the protection and propagation of a balanced indigenous population (BIP) of shellfish, fish, and wildlife, and to allow recreational activities. Under Section 125.62, the establishment of a water quality monitoring program is required which to the extent practicable:

- Provides adequate data for evaluating compliance with applicable water quality standards

- Measures the presence of toxic pollutants which have been identified or are reasonably expected to be present in the discharge.

In order to meet these water quality monitoring requirements, receiving water and sediment sample stations need to be located in the vicinity of the ZID boundary, at control sites, and in impact areas in such a way as to allow adequate correlations to be made between water quality, oceanographic and sediment measurements, and toxic substances and biological data. Other locations which a state may wish to specify include the shoreline in swimming and shellfishery areas and within the ZID. Placing stations so that pollutant concentration gradients can be detected between the ZID boundary and control stations may be valuable for larger discharges.

When a discharge is into a saline estuary there is a greater emphasis on protecting benthic organisms within the ZID, suggesting that water quality data near the seabed and sediment quality data may be necessary. In the case of oceanic discharges there are general requirements to prevent extreme adverse biological impacts within the ZID which have adverse effects beyond the ZID; thus, again some water quality monitoring within the ZID may be required for especially sensitive ecosystems and/or large or industrialized wastewater systems.

Criteria for selection of specific stations depend on the purpose of the station. ZID-boundary stations should be placed on the upcurrent and downcurrent boundaries of the ZID; they will not necessarily be at fixed locations but more likely will be set on the day of sampling based on observations of current direction. Since the objective is to intercept the waste field drift flow as it is carried across the ZID boundary, several samples placed at depth and across the wastefield need to be obtained. This will be necessary in order to compute an average value and to show a range of values if the waste field is not uniform. To demonstrate that the plume has indeed been sampled, especially if this is not evident by the water quality values themselves, data on currents, drogue tracks, and/or tracers need to be provided.

Stations upcurrent of the ZID may be the choice for water quality control stations, although with the gradient concept in mind, stations sufficiently far downcurrent may be satisfactory. Care should be taken in selecting control stations so that values presumably representing control conditions do not include diluted wastes carried back into the area of the control station by tidal currents. Control stations should be unaffected by other pollutant sources as well as the applicant's discharge. Also, control stations should be located in water of similar depth as the discharge, with similar bottom characteristics and similar distances from shore. Additional controls may be needed when the applicant's proposal is for a relocated outfall and when the discharge is into stressed waters.

Impact area stations vary from one discharge site to another. Areas where monitoring may be required include recreational beaches, diving areas, shellfish harvesting areas, kelp beds, coral reefs, commercial and recreational fishing grounds, and other distinctive biological habitats. The selection of impact area stations should be based upon a thorough review of the recreation and biological sections in the 301(h) application, the Technical Evaluation or Review Report, the tentative decision document, and the draft 301(h) permit. State requirements on station locations need to be met by the program (e.g., both shore and nearshore stations must be sited to protect beaches under the California Ocean Plan).

Additional stations may need to be located near other pollutant sources to allow the effects of the subject discharge to be distinguished from these sources. Examples of other pollutant sources are areas off the mouths of major rivers near the discharge, sludge disposal areas, and other municipal and industrial ocean discharges. The need for these stations is identified by noting the extent of influence from available water quality data, from analysis of potential impacts based on volumetric flow and characteristics of the discharge, and from analysis of the dispersion characteristics of the receiving water body.

All sited stations should be plotted on large-scale nautical charts or 15-min quadrangle sheets (USGS) and then transferred to more convenient small scale maps. Latitude and longitude should be determined from the large maps to the nearest second. The approximate depth at each station should be determined from previous sampling data or estimated from soundings shown on the large-scale charts. Each station location should be described relative to the outfall/diffuser, permanent navigation buoys, or distances from known shoreline points as shown in Table 4. Historical designations and/or the applicant's designation should also be noted. If ZID boundary stations are occupied using accurate navigation methods there should be adequate assurance that the resulting water quality sampling data reflect ZID boundary conditions. To document station locations, the applicant's program and periodic monitoring data reports should describe navigation (locating) methods and field conditions during sample collection.

Variables and Sampling Frequencies

The variables to be sampled in the receiving water include those specified in the 301(h) regulations, those required by the state, and those necessary to evaluate other water quality data. The variables which should be included routinely are BOD_5, dissolved oxygen, pH, temperature, salinity, suspended solids or its surrogates (e.g., light transmittance), total and fecal coliform bacteria, and settleable solids. Light transmittance may be specified in terms of turbidity, Secchi disc depth, extinction coefficient, or percent light transmittance. The applicant should state the reason(s) for the light transmittance method(s) selected. Additional variables which may be required are:

- Total nitrogen
  --nitrate
  --nitrite
  --total kjeldahl nitrogen
  --ammonia

- Total phosphorus
  --reactive phosphorus

- Chlorophyll a

- Floating particulates

- Color.

TABLE 4. EXAMPLE OF STATION LOCATION DESCRIPTIONS FOR 301(h) COMPLIANCE MONITORING

Station Number	N Latitude	W Longitude	General Description	Historical Designation	Approximate Depth, m[a]
1	21°15'10"	157°49'53"	3.14 km directly south of Waikiki Beach, approx. 2.4 km west-southwest of Diamond Head Beach Park.	None	70
2	21°17'44"	157°52'34"	0.79 km directly south of Sand Island in Honolulu Channel, along west edge.	2[b]	12.5
3	21°17'37"	157°53'22"	at end of old sewer outfall, 1.07 km offshore from Sand Island.	3[b]	12.8
4	21°17'46"	157°53'59"	along eastern edge of Kalihi Channel, 1.0 km directly southwest of coral reef, 0.7 km directly west of diffuser.	4[b]	12.8
5	21°17'44"	157°54'25"	2.74 km directly southeast of Ahua Pt., 0.66 km directly west pf Kalihi Channel, 0.60 km directly south of Keehi Lagoon coral reef.	5[b]	8.5
6	21°17'01"	157°54'24"	at the center of the zone of mixing 48 m north of diffuser within the ZID.	None	68
7	21°17'01"	157°53'59"	≃0.7 km directly east of center of zone of mixing, at the eastern edge of ZID.	None	70
8	21°16'56"	157°54'24"	130 meters directly south of center of zone of mixing at ZID boundary.	None	79
9	21°17'01"	157°55'00"	1.024 km directly west of center of zone of mixing, just outside west edge of zone of mixing.	S1-9[c]	128
10	21°17'16"	157°54'24"	at north edge of zone of mixing, 0.48 km directly north of center of zone of mixing, 0.53 km north of diffuser.	S1-6[c]	31
11	21°17'39"	157°54'57.5"	2.33 km south-southeast of Ahua Pt., 1.56 km directly west of Kalihi Channel, 0.83 km directly south of Keehi Lagoon coral reef.	None	15
12	21°17'08"	157°53'22"	0.91 km directly south of old sewer outfall, 1.77 km east of center of zone of mixing, 1.81 km directly south of coral reef at south end of seaplane runway.	None	70
13	21°17'22"	157°55'18"	2.68 km south of Ahua Pt., 1.69 km west-northwest of center of zone of mixing, 1.54 km directly south of Keehi Lagoon coral reef.	None	70
14	21°17'54"	157°55'46"	1.64 km directly south of Keehi Lagoon Beach, 2.86 km directly northwest of center of zone of mixing.	None	18
15	21°17'27"	157°52'26"	1.19 km directly south of southernmost tip of Sand Island, approx. 500 m south-southeast of entrance to Honolulu Channel.	None	18
16	21°15'11"	157°49'01"	0.76 km southeast of Diamond Head Beach Park near lighthouse and Coast Guard Res.	1[b]	18
17	21°17'01"	157°53'48"	1.0 km directly east of center of zone of mixing, just outside eastern edge of zone of mixing.	None	70

[a] All depths are relative to MLLW.

[b] Applicant's proposed monitoring station numbers.

[c] Historical sampling stations of applicant.

Water column profiles for salinity and temperature are needed to interpret dissolved oxygen data and may be necessary to predict the location of the drift flow unless profiling with tracers or effluent constituents is to be relied upon for finding the plume. In any event, salinity and temperature may be needed, together with current speed and direction, to aid in describing mass movement of diluted wastes to farfield sites where impacts must be assessed. Current directions are needed to determine where in the horizontal plane one might expect to find the drift plume crossing the ZID boundary. Current speeds are important in assessing the vertical height of plume rise and, hence, in establishing where in the vertical plane samples should be taken to measure plume constituents. Salinity, temperature, and currents, furthermore, need to be provided to assist in evaluating the results of benthic and other biological responses. In estuaries, the amount of freshwater inflow from rivers needs to be documented as an adjunct to evaluating residence times and routes of possible transport of diluted effluents and particulates. Sampling may be at generally accepted depths, e.g., 1 m below the water surface, mid-depth, 1 m above the sea bed and at 10-m intervals for depths greater than about 40 m; however, features of water masses observed in profiling for salinity and temperature (and possibly light transmittance) should take precedence in establishing sample depths.

Parameters to be measured in the sediments should include particle size distribution and total volatile solids. Other variables, such as BOD_5, sulfides, and total organic carbon, may be required by the states or may be important in analyzing discharge impacts on benthic biota. In addition, sediment samples should be analyzed annually for toxic substances and pesticides identified in the plant effluent. Sediment samples for toxic substance analysis should be taken within the ZID, in the vicinity of the ZID boundary, at representative impact area locations outside the ZID boundary, and at control stations.

Sampling in estuaries should be conducted at slack water as recommended in the Revised Section 301(h) Technical Support Document (Tetra Tech 1982). Where tidal effects are to be discriminated, sampling should be done at several times over a tidal cycle for both spring and neap tides. In order to verify continuing compliance with 301(h) criteria, the ZID boundary stations should be sampled during those times of year when the discharge is least diluted.

Sampling frequencies must be selected to meet state requirements and should provide data during the critical environmental period(s) as identified in the 301(h) application. For sites where available data do not define these periods adequately, receiving water sampling should be done monthly for at least the first year. In most other cases, quarterly surveys which cover the critical period(s) should suffice. More frequent sampling may be specified by the states in swimming and shellfishery areas to determine compliance with bacteriological standards.

Sampling and Analytical Methods

The monitoring programs should specify sample collection, preservation, storage, and analysis methods which are approved by the EPA and state agencies and are appropriate for the site. In addition to specification of analytical methods, the minimum accuracy, limits of detection, and desired number of significant digits to be recorded should be specified to help ensure that accurate and precise data are obtained. Receiving water samples should be taken with a Van Dorn, Frauchy, or comparable sampler and then transferred to the proper type of container. When variables are measured by electronic probe (e.g., temperature, conductivity, dissolved oxygen, and pH) values should be measured at 1- to 3-m (3- to 10-ft) intervals. Electronic probe systems often have severe accuracy problems. Frequent calibration is essential.

Sediment samples for organic carbon should include only the upper 2 cm (0.8 in) of the sediment to ensure that the sediment oxygen demand per unit mass is not diluted by underlying, stabilized, or inorganic material.

Table 5 lists sample preservation and storage requirements for some variables, showing minimum sample volume, type of container, preservative required, and maximum storage time. Any deviations in container type or preservative from those specified in this table should be noted on sample bottles, field data sheets, and in the monitoring reports. The volumes given are intended as minimum amounts. Sample volumes should be increased depending on the number of sample splits to be made.

TABLE 5. RECOMMENDED SAMPLE PRESERVATION AND STORAGE REQUIREMENTS FOR WATER QUALITY

Parameter	Volume Required (ml)	Container	Preservative	Holding Time
Oil and Grease	1,000	G only	Analyze immediately or 5 ml HCl at time of collection. Cool, 4° C	24 hours
Total Suspended Solids	Sufficient aliquot to contain residue of $\geq$ 25 mg. Successive aliquots of sample may be added to the same dish.	G, P	None. Analyze immediately.	No holding
Settleable Solids	1,000	P, G	None	24 hours
Extinction Coefficient	100	P, G	Analyze same day or cool, 4° C	7 days
pH	25	P, G	Determine on site or cool, 4° C	6 hours[a]
Ammonia Nitrogen	400	P, G	Cool, 4° C H_2SO_4 to pH <2	7 days
Salinity	240	G (with paraffined corks)	None	1 hour (Longer if properly sealed air tight)
Temperature	1,000	P, G	Det. on site	No holding
Biochemical Oxygen Demand	1,000	P, G	Cool, 4° C	24 hours
Total Phosphorus	50	P, G	Cool, 4° C H_2SO_4 to pH <2	24 hours
Reactive Phosphorus	50	P, G	Filter on site, Cool, 4° C	24 hours
Nitrate - N	100	P, G	Cool, 4° C H_2SO_4 to pH <2	24 hours >24 hours
Nitrite - N	50	P, G	Cool, 4° C	24 hours
Total Kjeldahl Nitrogen	50-500	P, G	Cool, 4° C H_2SO_4 to pH <2	24 hours
Dissolved Oxygen	300	G only	Determine on site	No holding
Color	50	P, G	Cool, 4° C	24 hours
Total and Fecal Coliform	100	P, G, sterilized	Add Na thiosulfate to effluent samples	Analyze in field or 6 hours
Total Organic Carbon	25	P, G	Cool, 4° C H_2SO_4 to pH <2	24 hours
Chlorophyll a	200	P, not acid-washed, keep in darkness away from light	Filter on site, add $MgCO_3$ during filtration	Process immediately or 2 weeks if frozen and kept in dark.

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[a] If samples cannot be returned to the laboratory in less than 6 hours and holding time exceeds this limit, the final reported data should indicate the actual holding time.

References: Standard Methods (American Public Health Association 1980) and Methods for Chemical Analysis of Water and Wastes (U.S. EPA 1979b).

Chemical analysis procedures for sediments are basically the same as for water samples once the sediments have been digested. The EPA/Corps of Engineers manual, Procedures for Handling and Chemical Analysis of Sediment and Water Samples (Plumb 1981), should be consulted for detailed guidance on sediment sample handling and digestion procedures. Sediment samples can be stored dried, frozen, or on ice for metals analyses. If analysis of organic constituents is to be performed, sediment samples should be stored on ice only.

Analytical methods should be selected based on the water quality standards and the EPA-approved methods listed in 40 CFR Part 136, with due consideration of the extent to which interferences occur in the receiving water and wastewater samples. When several methods are available, the selection should be made by comparing the accuracy and precision of the candidate methods for the concentration range expected at the site, and the adequacy of the detection limit of each method relative to the pertinent water quality standard. Table 6 shows recommended methods for the same variables listed in Table 5 along with acceptable minimum precision and detection limits for each method. The table also shows the desired number of significant digits to be reported for each parameter.

Oceanographic Measurements

Oceanographic measurements to meet the 301(h) objectives include two parts: data needed to detect plume and sediment movement, and observations needed to interpret the water quality and biological data. This section discusses acquisition of current, wind, and tide data. Verification of initial dilution calculations is not required. However, if plume calculations are considered by the applicant to be unreliable or inaccurate, it may be desirable to obtain supplemental monitoring data to improve the models or to document field validation of other models.

The POTW's 301(h) application and its Technical Evaluation or Review Report should be reviewed to determine if available current data and knowledge of wind and tide effects are adequate to determine the direction of movement of the wastefield beyond the ZID boundary, the subsequent dilution, and the direction of movement of the sediment from the discharge.

TABLE 6. RECOMMENDED ANALYTICAL METHODS

Parameter	Method	Precision	Minimum Detection	Significant Digits Desired
Flow	Continuous measurement, automatic device	± 8 percent	0.02 MGD	3
Grease and Oil	Gravimetric, Separatory Funnel Extraction EPA Method 413.1	± 0.9 mg/l N/A[a]	5 mg/l mg/m²	2
Floating Particulates	Flotation funnel extraction	N/A	mg/m²	2
Total Suspended Solids	Gravimetric, Dried at 103-105° C. EPA Method 160.2 (SM, 14th ed., p. 91, Section 208D)	approx. ± 5 mg/l[b]	10 mg/l	2
Settleable Solids	Volumetric, Imhoff Cone. EPA Method 160.5. Gravimetric Method (SM 14th ed., pp. 95-96, Sec. 208F)	N/A	1 ml/l/h	2
Extinction Coefficient	Light transmissometer	N/A		2
Discoloration	Presence or absence of color at surface	N/A	N/A	N/A
pH	Potentiometric. EPA Method 150.1 (SM 14th ed., p. 460, Sec. 424)	± 0.1 standard unit	pH = 12	3
Salinity	Induction Salinometer or titration. (SM 14th ed., p. 107, Sec. 209C)	titration: ± 0.05 ppt	1 ppt	4
Temperature	Bathythermograph or Thermometric. EPA Method 170.1. (SM 14th ed., p. 125, Sec. 212)	± 0.05° C	--	3
Dissolved Oxygen	Modified Winkler, Full Bottle Technique, with azide modification for effluent sampler or Membrane Electrode when calibrated with Modified Winkler with azide modification for effluent samples. EPA Method 360.2 or EPA Method 360.1 when calibrated with EPA Method 360.2 (SM 14th ed., pp. 441-447, Sec. 422 A and B for Winkler; SM 14th ed., p. 450, Sec. 422F for probe)	± 0.05 mg/l (Winkler) Probe: ± 0.1 mg/l	0.1 mg/l 0.1 mg/l	3
Biochemical Oxygen Demand	5 day, 20° C EPA Method 405.1 (SM 14th ed., p. 543, Sec. 507)	± 0.7 mg/l BOD at 2 mg/l BOD ± 26 mg/l BOD at 175 mg/l BOD	1 mg/l	2
Chemical Oxygen Demand	EPA Method 410.3 (SM 14th ed., p. 550, Sec. 508)	± 13 mg/l COD	1 mg/l	2
Total Organic Carbon	Combustion-Infrared Method EPA Method 236 (SM 14th ed., p. 532, Sec. 505)	N/A	N/A	2
Ammonia Nitrogen	Automated Phenate Method. EPA Method 350.1 (SM 14th ed., p. 616, Sec. 604 or Strickland and Parsons, p. 67)	± 0.005 mg NH_3-N/l	0.01 mg NH_3-N/l	2
Nitrate-Nitrogen[c]	Technician Auto Analyzer II or Spectrophotometric, manual, Cadmium Reduction. EPA Method 353.2 or EPA Method 353.3. (SM 14th ed., p. 423, Sec. 419c, for manual method)	Automated, Cadmium: ± 0.092 mg/l at 0.35 mg/l Spectrophotometric, Cadmium: ± 0.004 mg/l at 0.24 mg/l	0.05 mg/l	2

TABLE 6. (Continued)

Parameter	Method	Precision	Minimum Detection	Significant Digits Desired
Nitrite-Nitrogen	Technician Auto Analyzer II or Diazotization. EPA Method 354.1 (SM 14th ed., p. 434, Sec. 420)	N/A	0.01 mg NO_2-N/l	2
Total Kjeldahl Nitrogen	Technician Auto Analyzer II or Colorimetric, EPA Method 351.3. (SM 14th ed., p. 437, Sec. 421)	Colorimetric ± 1.056 at 4.10 mgN/l	Colorimetric <1 mgN/l	2
Total Phosphorus	Technician Auto Analyzer II or Digestion, Manual Ascorbic Acid Technique. EPA Method 365.2 (SM 14th ed., p. 476, Sec. 425C(III) and p. 481 Sec. 425F for manual method)	Automated: ± 0.130 mgP/l at 0.8 mgP/l Manual: ± 0.033 mgP/l at 0.11 mgP/l	0.005 mgP/l	2
Fecal Coliform	Multiple Tube Fermentation Technique, MPN Test. (SM 14th ed., p. 922, Sec. 908C)	MPN with 95 percent confidence limit	NA	2
Total Coliform	Multiple Tube Fermentation Technique, MPN Test (SM 14th ed., p. 916, Sec. 906A) or for seawater only Membrane Filter (SM 14th ed., p. 928, Sec. 909)	MPN with 95 percent confidence limit	NA	3
Chlorophyll *a*	Spectrophotometric (SM 14th ed., p. 1029, Sec. 1002G Strickland and Parsons, SCOR/UNESCO Equation, pp. 185-194)	NA	NA	2
Particle Size Distribution[d]	Sieve Analysis (Buchanan)			

[a] N/A = not available.

[b] Minimum accuracy, when given as a range about a specific value, has been taken from the below listed references. The associated ranges are for 1 standard deviation about the mean value.

[c] The cadmium reduction method determines nitrate + nitrite-nitrogen. The nitrate-nitrogen is calculated by subtracting nitrite-nitrogen as determined by a separate diazotization test.

[d] Detailed procedure is given in the biological monitoring section.

References:

Buchanan, J.B. 1971. Sediments. In: International Biological Program (IBP) Handbook No. 14. Blackwell Scientific Publ., Oxford, pp. 30-52.

CLMBS: Great Lakes Region Committee on Analytical Methods. 1969. Chemistry Laboratory manual bottom sediments. EPA, Federal Water Quality Administration, Washington, D.C., 101 pp.

EPA Method: U.S. Environmental Protection Agency. 1979b. Methods for Chemical Analysis of Water and Wastes. USEPA, Environmental Support Laboratory, Cincinnati, OH.

SM 14th ed.: American Public Health Association. 1980. Standard methods for the examination of water and waste water. 14th ed., Washington, D.C. 1193 pp.

Strickland and Parsons: Strickland, J.D.H., and T.H. Parsons. 1972. A practical handbook of seawater analysis. Dilution 167, 2nd ed. Fisheries Research Board of Canada. Ottawa, Canada. 310 pp.

The current data should be reviewed to determine whether surface, bottom, and mid-depth currents were measured. The sampling times should be reviewed to determine whether periods of minimum and maximum stratification and other important conditions (e.g., onshore winds, upwelling periods) were covered.

Winds generally are an important influence on coastal currents. Current data should therefore be checked to see if historical surface currents were measured concurrent with wind measurements and to what depth wind effects are discernible. The wind data should be reviewed to estimate frequency of occurrence of onshore transport by season and location. This information is helpful in identifying discharge impact areas along the shoreline. Any deficiencies in available current data should be noted and a determination made as to whether intensive current monitoring is needed or if specific data gaps need to be filled.

Field observation methods selected for oceanographic measurements depend on the kinds of information needed, the extent of potential discharge impacts, the oceanographic and physical conditions at the site, and resources available to the applicant. Drogues, drifters, or dye released from the outfall site may be used to determine mean current velocities at specified depths, and also to provide information on the direction of movement and the dispersive properties of the velocity field. These studies may also identify nearshore eddy patterns or "dead" circulation zones which may be present. Drogues set just above the bottom, or seabed drifters, can be used to determine the direction of sediment movement.

Oceanographic data which should be recorded at the time of water quality and biological sampling include:

- Wind speed and direction
- Sea state (height of swell and waves).

A variety of field study methods are available to collect oceanographic information. The appropriate use and limitations of current meters, drogues and drifters, dye studies, and field positioning methods are discussed in Appendix A to this document.

Data Analysis and Reporting

Data reporting procedures include the preparation of field logs, sample container labels, laboratory data sheets, and reporting forms. The reporting forms should be completed and sent to the appropriate U.S. EPA Regional Office on the schedule prescribed in the 301(h) permit. Chain of custody forms, showing the transfer of data from the field to the laboratory, and finally to the U.S. EPA, should be maintained along with field logs and laboratory data sheets.

The type of information recorded on the field logs and sample labels should ensure that samples are identified properly and data are recorded accurately. Field logs should include station location and number, depth of samples, type(s) of samples taken, date and time of sampling, surface observations as specified in the oceanographic section, depth of water at the station where samples are taken, all field water quality measurements, and the names of all individuals who collected the samples. This information should be entered on the field log at the time of sampling. The sample container labels should give sample number, station location, and number; date, time, and depth of sample; treatment of sample (e.g., H_2SO_4 added); a code designating what analyses are to be done on the sample, and the name of the individual(s) collecting the sample. Laboratory data sheets should include sample number, station location and number, sampling date and time, and name of the analyst. For each individual analysis, results should be reported along with the unit of measurement, duration of sample storage, date sample was analyzed, and any comments on deviations from laboratory procedures or unusual sample conditions. The chain of custody forms should show the name of the person to whom the form is being sent; and the name of the person receiving the data and date received.

Receiving water quality and sediment data should be compared with NPDES requirements (when applicable) and applicable water quality standards. Spatial gradients should be examined to determine whether elevated concentrations occur near the outfall and, if so, where concentrations return to background levels. Analysis of temporal trends should be done to identify seasonal differences. Appropriate statistical tests (e.g., ANOVA) can be used to determine if statistically significant differences exist between the ZID-boundary and reference (control) stations. The water quality and sediment data can be used to define and report on the spatial extent of the wastewater plume and sediment deposition area. This information should be used in conjunction with the biological monitoring data to identify and interpret any changes detected in the biota.

CHAPTER IV

BIOLOGICAL MONITORING

OBJECTIVES

Biological monitoring is necessary to evaluate the overall impact of the permittee's modified discharge. The primary objective of the biological monitoring program is to provide evidence that:

- There is a continued attainment or maintenance of water quality which assures protection and propagation of a balanced, indigenous population (BIP) of shellfish, fish, and wildlife beyond the zone of initial dilution (ZID) and in the vicinity of the ZID boundary

- Conditions within the ZID do not contribute to extreme biological impacts, such as the destruction of distinctive habitats of limited distribution (e.g., kelp beds and coral reefs), the presence of disease epicenters, or the stimulation of phytoplankton blooms which have adverse effects beyond the ZID, etc.)

- For discharges into saline estuarine waters: a) benthic populations within the ZID do not differ substantially from balanced, indigenous populations which exist in the vicinity of the ZID boundary, b) the discharge does not interfere with estuarine migratory pathways within the ZID, and c) the discharge does not result in an accumulation of toxic pollutants or pesticides at levels which exert adverse effects on the biota within the ZID

- There is a continued attainment or maintenance of water quality which allows for recreational activities (including fishing) beyond the ZID boundary and such activities in the vicinity of the modified discharge are not restricted unless such restrictions are routinely imposed around sewage outfalls discharging secondary effluent.

Design of the biological monitoring program requires careful consideration of potential impacts specific to the 301(h) permitee's discharge(s). Factors which are important to designing biological monitoring programs and individual sampling procedures are discussed below.

APPROACH AND RATIONALE

Section 125.62(b) of the amended 301(h) regulations requires that the biological monitoring programs for both small and large 301(h) discharges must provide date adequate to evaluate the impact of the modified discharge on the marine biota. This generally necessitates comparing the characteristics of selected marine communities in the vicinity of the discharge with the characteristics of similar communities in reference areas. Therefore, the same type of comparative strategy required for demonstrating a balanced, indigenous population (BIP) of shellfish, fish, and wildlife in the application should be incorporated into the biological monitoring program. [See the Revised Section 301(h) Technical Support Document (Tetra Tech 1982) for guidance on demonstrating a BIP in a 301(h) application.]

Under Section 125.62(b)(1)(i-iv) of the amended regulations, biological monitoring programs must to the extent practicable include:

- Periodic surveys of the biological communities and populations most likely affected by the discharge, as well as those at suitable control sites, to enable comparisons with baseline conditions

- Periodic determinations of the accumulation of toxic pollutants and pesticides in organisms and examination of adverse effects such as disease, growth abnormalities, physiological stress, or death

- Sampling of sediments in the vicinity of the ZID boundary, in other areas of expected sediment accumulation, and at appropriate reference sites to support water quality and biological surveys and to measure the accumulation of toxic pollutants and pesticides

- Periodic assessment of the conditions and productivity of commercial or recreational fisheries where the discharge would affect such fisheries.

Except for the periodic survey requirement, small permittees are not subject to these specific requirements if they discharge at depths greater than 10 m and can demonstrate through a suspended solids deposition analysis that there will be negligible sea bed accumulation in the vicinity of the modified discharage. However, small permittees still must provide adequate data to evaluate the impact of the modified discharge on the marine biota. This should involve the establishment of a background data base and the demonstration of predicted biological impacts of the small discharge. In all cases, site-specific characteristics will affect the selection of the number of sampling sites, sampling locations, and the required sampling effort in each biological category.

Information available in the discharger's 301(h) application and in other investigations conducted near the discharge should be utilized fully to identify physical-chemical and biological characteristics of the potentially affected receiving waters. Characterization of the oceanographic and meteorological setting of the discharge area will be necessary to make decisions concerning positioning of the discharge and reference sampling stations. Available biological data should be reviewed to define limits of natural variability in biological populations. The number of sampling stations and number of replicate samples at each station should be determined, in part, on the basis of this information. In these respects, the historical data may serve the same purposes as a pilot survey. Decisions concerning taxonomic groups to be sampled, station locations, types of sampling equipment, sample handling, sorting procedures, and ancillary measurements of physical-chemical parameters should be made on the basis of existing information to the extent practicable. Where effects of the proposed discharge on specific biological communities or important species has not been clearly resolved, the monitoring program should be designed to fill such data gaps.

In designing the program specifications, the complementary nature of the water quality and biological monitoring programs should be recognized. Concurrent collection of biological and water quality information should be emphasized in an effort to identify causal relationships.

SPECIFICATIONS FOR BIOLOGICAL MONITORING

Sampling of Biological Communities

The 301(h) regulations require "periodic surveys of the biological communities and populations which are most likely affected by the discharge to enable comparisons with baseline conditions described in the application." Emphasis should generally be placed on monitoring of benthic communities due to the inherent community characteristics, sampling considerations, and the importance of the benthos in the marine ecosystem. Benthic communities adjacent to pollution sources can generally provide information on the areal extent of impact more readily than other biological communities because many benthic organisms are sedentary or relatively immobile and are, therefore, continually exposed to pollution stress. Benthic communities are also more easily sampled than other biological communities and benthic sampling methods are more standardized than methods for other communities. Existing information on benthic communities is often sufficiently extensive to provide documentation of both the magnitude and direction of the community response to specific perturbations. Finally, benthic communities are of a primary importance in the food chain of the nearshore marine environment. For the above reasons, monitoring of the macrobenthos should normally be a primary element of 301(h) permit biological monitoring programs.

Another principal monitoring requirement defined in the regulations is periodic assessment of the accumulation of toxic pollutants and pesticides in the biota. These assessments are required as part of a specific monitoring effort for measuring the impact of elevated or increasing levels of toxic pollutants and pesticides on susceptible biological communities. Bivalves (e.g., *Mytilus californianus* and *M. edulis*), have been shown to greatly concentrate most identified marine pollutants relative to ambient concentrations in seawater. Because water quality characteristics (e.g., temperature and dissolved oxygen) affect biological uptake, the concentrations of toxic substances in the tissues of these organisms will more accurately reflect the site-specific potential for bioaccumulation than will the measurement of ambient concentrations of toxic substances. For these reasons, caged bivalves used in offshore biomonitoring systems may provide an early warning of excessive water column contamination, and may be used to monitor the potential for transfer of toxic pollutants and pesticides into and through the food chain. Such an in situ biomonitoring system also provides a means of evaluating the effectiveness of toxic control programs.

Monitoring program requirements also include the periodic assessment of commercially or recreationally important fisheries that may be affected by the discharge. The objective of fisheries monitoring is to assess the condition and productivity of those fisheries. Fisheries monitoring may consist of the periodic review of catch data collected by state agencies, interviews of sport fishermen to determine success rates, or field and market sampling of the fish or shellfish populations.

The biological monitoring program regulations specify that the permittee monitor the biological communities and populations which are likely to be affected by the discharge using comparisons with baseline conditions described in the application. In addition to the benthos, such communities may include phytoplankton, zooplankton, and fishes.

Numerous site-specific characteristics of the environment may necessitate additional biological monitoring. For example, hydrographic characteristics (current patterns, water residence time) and nutrient concentrations in an estuary or embayment may result in the potential for long-term biological changes such as eutrophication. If such changes are determined to be a potential impact of the modified discharge, periodic monitoring of the phytoplankton community may be required. Furthermore, if changes in species composition of the phytoplankton occur and thereby induce changes in the species composition of the herbivore community, zooplankton monitoring may be required.

In certain geographic settings, some coordination of the monitoring programs of adjacent dischargers may be required so that those dischargers periodically sample the same biotic groups. These conditions include, for example, the situation where the potential for eutrophication exists, or where further examination of the multiplicative effects of several dischargers on widespread toxic dinoflagellate blooms is needed.

Monitoring should be initiated to ensure the continued existence of distinctive habitats of limited distribution. Such habitats include, but are not limited to, kelp beds, coral communities, and rocky intertidal communities.

Station Locations

To meet the minimum requirements of the biological monitoring program, sampling of the selected biological communities in the vicinity of the ZID, in any other areas of expected impact, and at appropriate control sites will generally be required. Other sampling locations which may be specified include nearfield areas where important habitats have been identified, and also at both new and old discharge sites in the case of an improved discharge involving outfall relocation. In the case of large permittees, additional sampling is recommended at intermediate locations between the ZID boundary and control stations along a gradient of effluent concentrations to help define the spatial extent of biological effects.

Information derived from the water quality monitoring program will be important in interpreting the results of biological sampling. Therefore, the selection of water quality and biological stations must not be done independently. In addition, the station selection process of the biomonitoring program should place emphasis on the inclusion of historical sampling sites. This will maintain sampling continuity, and additional information will thus be made available for impact assessment.

In instances where chemical analysis of the effluent and/or sediments in the vicinity of the discharge has identified toxic pollutants at levels of concern, the required bioaccumulation monitoring of toxic substances should be undertaken in the vicinity of the ZID boundary, at other areas of expected impact, and at control site(s). Test organisms utilized for in situ biomonitoring studies should be placed at the depth of the plume during the exposure period. If the plume surfaces, exposure should be conducted at a sufficient distance below the surface to prevent damage to or loss of the exposure apparatus. Additional exposure depths may be used if the plume depth is uncertain or variable, or if past discharges have resulted in substantial sediment contamination. In cases of past sediment contamination, near-bottom exposures can be used to evaluate the contribution of sediments to bioaccumulation levels.

The Technical Evaluation Report, the tentative decision document, and the NPDES [301(h)] permit should be reviewed to determine requirements for sampling other biotic groups. If this review indicates the need to sample phytoplankton, zooplankton, or fish communities, sampling locations should be specified in the vicinity of the ZID boundary, other areas of expected impact, and at one or more control sites. The number of control sampling locations, as well as the need for and number of any sampling sites specified in nearfield areas, should be determined based on the nature of the potential impacts of the discharge. As in the example previously presented, sampling depths should be specified for each of the above sampling station groups. Determination of sampling depths must be made on the basis of oceanographic conditions and behavioral characteristics of the organisms; these depths may vary seasonally. The monitoring of habitats of limited distribution may also be required in the nearfield area at a single site or at several locations including control sites.

There would be additional station requirements for discharges into stressed waters or in situations where other pollutant sources could potentially affect biological communities in the vicinity of the applicant's discharge. In such cases, it is important to define the magnitude of the discharge interaction(s) and describe any biological response gradients associated with the applicant's discharge and other pollutant sources in the study area. Therefore, several additional stations may be required at intermediate positions between the applicant's discharge and other significant pollutant sources in the study area.

For cases where there is an improved discharge involving outfall relocation, monitoring is required in the vicinity of the ZID boundary at both the relocated discharge site and at the existing discharge site until discharge at the latter site ceases. Within-ZID stations, if necessary, should be located close to the midpoint of the diffuser. ZID-boundary stations should be oriented in the direction of the predominant current. Single within-ZID and ZID-boundary stations should be sufficient for small discharges, while two or more stations of each type may be needed for larger discharges.

Selection of control stations is one of the more important aspects of the design of the monitoring programs, since all assessments of impacts will rely on comparisons made with data from these locations. Control stations should be located outside the traceable waste field and not be affected by the applicant's discharge. Similarly, the selected locations should not be influenced by other discharges. Control stations should be located in water of similar depth to that of the within-ZID, ZID-boundary, and gradient stations. Sediment characteristics should be similar at all sampling stations except where sediment alterations are due to an outfall effect. Control and other monitoring stations should be located approximately the same distance from shore. Since it is often necessary to locate control sites a considerable distance [5-10 km (3-6 mi)] from the outfall to escape all waste field influences, candidate control sites should be carefully evaluated to ensure that oceanographic conditions are not atypical.

Example layouts of sampling locations for two alternative biological monitoring programs are presented in Figure 1. Included in the example are sampling stations that should be expected at a relatively large (x stations) or small (o stations) discharge. Some of the important features common to both layouts that should be noted are: sampling stations have been located at the same depth and at approximately the same distance from shore; near-ZID and nearfield gradient stations are positioned in the same direction from the diffuser as the predominant current direction; and, control stations are located a considerable distance from the diffuser and in the opposite direction of predominant currents.

Although type(s) of stations are not specified in the figure, the layouts are typical of expected benthic sampling designs. Sampling of other biotic groups, if required, might also be conducted at these stations. The number and location of stations are indicative of the most basic programs that would be expected of relatively small and large discharges. In the

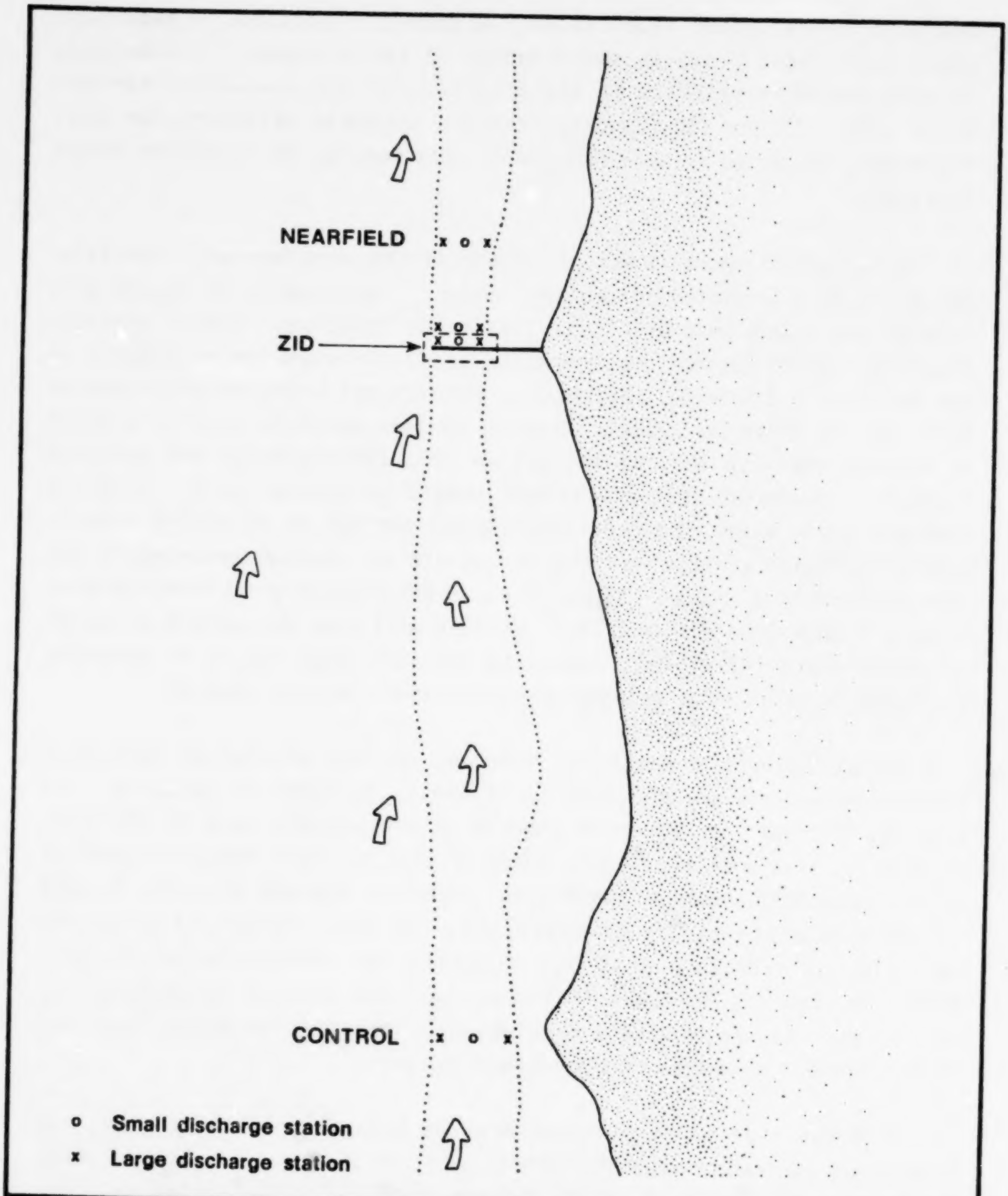

NEARFIELD
ZID
CONTROL
o Small discharge station
x Large discharge station

Figure 1. Representative sampling locations for two levels of biological monitoring.

case of a large discharge where a high potential for sediment accumulation offshore has been identified, a number of stations would be required in the deeper water offshore of the diffuser and at appropriate control stations. These example layouts also do not reflect the existence of areas of special concern, such as important fish habitats where additional sampling might be required.

Sampling Frequency and Replication

The 301(h) regulations do not offer explicit guidance for either sample frequency or replication. For those biological communities likely to be affected by the discharge, sampling frequency will be dictated by community-specific characteristics. For example, due to the rapid response of phytoplankton to environmental perturbations and seasonal fluctuations in community structure, the most effective sampling strategy might be intensive sampling for relatively short periods of time. Similarly, the ability to sample juvenile fish in nursery areas may be limited to certain seasons of the year. These examples point to the fact that sampling strategies should be considered in the sampling design. In the development of the strategies, data should be reviewed carefully to consider life history characteristics of target species.

Sample replication requirements are both site- and species-specific. Decisions on the level of sample replication or sampling effort should include careful consideration of the minimum detectable difference in selected biological parameters. Field experiments should be planned carefully in order to define minimum detectable difference levels, to establish the number of replicate samples required, and to specify the appropriate analytical approach.

Prior to designing a sampling program, the applicant should consider two important criteria associated with the sensitivity of the study to changes in biological parameters. These are the probability of rejecting the null hypothesis when it is true (commonly called the probability, or Type I error) and the probability of accepting a null hypothesis when it is false (commonly called the probability, or Type II error). The complement of β $(1 - \beta)$ is referred to as the power of a test and is especially important since it defines the probability of correctly detecting experimental effects (e.g., differences among sampling stations).

For a specified variance associated with a biological parameter, the probabilities of α, β, and minimum detectable differences among sampling areas can be expressed as a function of sample size. The allocation of sampling resources (stations, replication, and frequency) can then be determined with regard to available resources, practicality of the design, and desired sensitivity of the subsequent analyses. Discussions and examples of this approach are included in Cohen (1977), Winer (1971), Scheffe (1959), Moore and McLaughlin (1978), Gordon et al. (1980), and Saila et al. (1976).

Sample Collection and Processing

The following subsections provide a discussion of appropriate sample collection, sample handling, and quality assurance/quality control methods for the individual biotic groups which may be included in a 301(h) monitoring program.

Benthos--

Most biological monitoring programs will emphasize the macrobenthos since micro- or meiofaunal benthic samples are difficult and expensive to process and also present interpretive difficulties due to extreme small-scale heterogeneity and lack of understanding of community relationships. Should impact upon micro- and meiofaunal benthos prove significant for some outfalls such that monitoring of these infaunal components is required, the investigators should consult Fenchel (1969), Wieser (1960), McIntyre and Murison (1973), and Hulings and Gray (1971) for information on sampling methods and sample handling.

The methods and equipment for sampling macrobenthic infaunal communities have been the subject of several publications [Holme and McIntyre (1971), Word (1976), Hedgpeth (1957), and Swartz (1978)]. The ideal bottom grab for sampling all sediment grain sizes, from sand to silt, has yet to be invented. Word (1976) compares the sampling efficiency of seven grab samplers (Ponar, corer, Shipek, van Veen, orange peel, Smith-McIntyre, and a chain-rigged van Veen) in the silty-sand to clayey-silt sediment off southern California. The results of Word's investigation suggest that a 0.1-m^2 chain-rigged van Veen grab is the most reliable sampling device for these sediment types. Swartz (1978) recommends the use of a 0.1-m^2 Smith-McIntyre grab due to its essentially constant "bite" area; however, he recognizes that the depth of penetration of this grab varies with sediment type.

Regional consistency in infaunal monitoring is important to the 301(h) program objective. In the southern California area, for example, sampling should consist of replicate sampling using a 0.1-m^2 chain-rigged van Veen grab. In more sandy areas, this grab or the Smith-McIntyre grab recommended by Swartz (1978) may prove acceptable; however, investigators studying sandy infaunal communities should define the sampling efficiency of whichever grab they chose to utilize.

In areas where visibility and oceanographic conditions permit, diver-operated coring or dredging may be more desirable than grab sampling from a surface vessel. The type and size of sampling device suitable for each kind of substrate may vary from suction dredges (Brett 1964; Gale and Thompson 1975) which cover large areas (for substrates with a low density of organisms) to small coring tubes or small box corers (for substrates with a fairly high density of infauna).

The number of replicate samples collected at each station should be sufficient to ensure statistical reliability (see Sampling Frequency and Replication above and Effect of Sample Size below). At each station, one or more additional, separate sediment sample(s) should be collected for analysis of total organic carbon content, grain size distribution, and percentage of gravel, sand, silt, and clay. Other physical-chemical parameters discussed in the water quality monitoring section of this report should be monitored at or near each benthic station.

Sample Handling--The monitoring design should describe all procedures used in the benthic sampling program. These descriptions should include the following requirements:

1. Each replicate sample should be screened and preserved in the field on the day of collection.

2. Each replicate sample should be screened, fixed, sorted, and processed separately.

3. Samples should be screened through a sieve having 1.0-mm mesh. If smaller mesh size screens are also used (e.g., 0.5 mm), the fraction of the organisms retained by the 1.0-mm sieve and smaller sieves should be processed separately.

4. Organisms should be fixed in a buffered 10-percent formalin-seawater solution. (Borax is suggested as a buffering agent.) The specimens should be transferred to a 70-percent ethanol solution after an initial fixation period of 24 hours to 1 week. Vital staining techniques may be used as an aid to sorting (see Holme and McIntyre 1971, Williams and Williams 1974).

5. Permanent labels should accompany each sample throughout all phases of sample handling, processing, and storage. These labels should include the date and time of collection, the station and replicate identification number, the station location including at least latitude and longitude, and the sample collection depth. If available at the time of sample collection, other label entries should include water temperature, salinity, dissolved oxygen, and bottom depth.

Sample Processing--

1. The organisms should be sorted and identified to species, or, if unidentifiable, sorted into discrete taxa.

2. The total number of individuals of each species (or lowest identified taxon) in each replicate should be determined. Counts should be expressed as the number of individuals of each taxon in the samples and per m^2.

3. The wet weight of organisms in the six major taxonomic groups (polychaetes, crustaceans, molluscs, coelenterates, ectoprocts, and echinoderms) and the total biomass of each replicate sample should be determined.

Effect of Sample Size--The accuracy and precision with which benthic community parameters are estimated depend on the parameter in question and on the size of the sample. It is therefore appropriate to discuss the effects of sample size on estimates of parameters most frequently used to describe benthic community structure and function.

The total area sampled among the replicates at each station should be large enough to estimate a given parameter within acceptable limits of accuracy and precision. Within a study area, adequate sample size may be determined by maximizing the number of species collected or by minimizing the error of estimation of the mean for the parameter in question (Gonor and Kemp 1978). Alternatively, the surface area sampled may be determined by a review of sample sizes which in past studies have been shown to yield data with acceptable limits of accuracy and precision. If the surface area sampled per station is too small, the data will poorly estimate the parameter in question because the ratio of the variance to the mean for a given parameter will be unacceptably large (Gonor and Kemp 1978). Consequently, within-habitat variability (which is a function of nonrandom distribution of the fauna) will obscure differences in community structure when stations are compared.

Holme and McIntyre (1971) and Swartz (1978) recommend that an area of 0.5 m^2 (5.4 ft^2) be sampled to assess species composition in coastal and estuarine regions. This recommendation is supported by the results of benthic studies in Puget Sound (Lie 1968). From an analysis of ten 0.1-m^2 (1.1-ft^2) replicates at one site, Lie concluded that a minimum of five replicates is needed to accurately assess species composition, while a minimum of three replicates is required to accurately estimate biomass and numerical abundance.

Word (1976) presented an analysis of 10 replicate samples from southern California (location not given). He observed that: 1) the cumulative number of species does not appear to approach an asymptote with increasing number of samples, and 2) a second sample will include newly acquired species which constitute only 10 percent of the individuals in the first and second samples. Word concludes that because numerical clustering strategies are sensitive to species which contribute 90 - 95 percent of the total number of individuals, a single 0.1-m^2 (1.1-ft^2) sample is sufficient to obtain "useful descriptive information" (Word 1976).

Although Word et al. (1980) has shown that a single 0.1-m^2 (1.1-ft^2) sample is appropriate to describe the Infaunal Trophic Index (ITI is a single number characterizing the trophic organization of soft bottom benthic communities) in southern California, the single sample limits the degree of community characterization. With only a single sample, there is no direct estimate of within-group variance for statistical analyses. Because individuals are distributed logarithmically among the species of a community (Preston 1948, Sanders 1968), the species collected in the second and successive replicates most often will be numerically "rare." Note that "rare" is not synonymous with "unimportant." Predators, for example, are usually "rare" because they are one trophic level removed from their prey; yet, predators are usually a major factor influencing the diversity, structure, and function of benthic communities (e.g., Connell 1961, Paine 1966, Bilyard 1974). Hence, it should be acknowledged that one 0.1-m^2 (1.1-ft^2) sample is generally not adequate to characterize benthic community structure and function. Many uniformly distributed "rare" species which are important in maintaining community structure and function will not be captured in a single sample. In general, then, five replicate samples per station are recommended for determining benthic community structure and function, unless evaluation of site-specific data indicates that sufficient sensitivity could be obtained with fewer samples or that a greater number is required due to extreme spatial heterogeneity.

The previous discussion concerns the number of replicates (or area sampled) generally required to adequately characterize infaunal communities. The other major aspect of sample size concerns the statistical sensitivity or power associated with the number of replicate samples. A discussion of the statistical aspects is included in a previous section (Sampling Frequency and Replication).

Quality Assurance and Control Procedures--To assure proper handling and processing of benthic samples, the following procedures are recommended:

1. At least 5 percent of all samples should be resorted by individuals different from those who conducted the original

sorting. Records on the results of this second sorting should be maintained and presented in an appendix to the monitoring report. This should be a double-blind test.

2. Complete sorting, processing, and/or laboratory records for each replicate sample should be included in a separate appendix volume to the annual report. These record sheets should present as a minimum the data specified in Item 5 of Sample Handling (above). The names and detailed statements of the qualifications of all persons performing and confirming taxonomic identifications of organisms should be included in the appendix volume.

3. A voucher collection consisting of specimens representative of each species (or lowest taxonomic unit of identification) collected during this monioring program should be developed and maintained by the applicant. This collection should be archived for a period of not less than 2 years after the expiration date of the 301(h) modification and should be housed at a facility where adequate curatorship can be assured.

4. Taxonomic references used for the identification of organisms should be cited in the appendix to the report.

Bioaccumulation Studies--

The amended 301(h) regulations require periodic determinations (except for small applicants meeting certain depth and solids deposition criteria) of the accumulation of toxic pollutants and pesticides in organisms and examination of other adverse effects of the discharge such as disease, growth abnormalities, physiological stress, or death. At discharges where bioaccumulation of toxic substances is known or likely to be a problem, tissue samples from resident macroinvertebrates and fish species should be examined for abnormal body burdens of toxic substances. The identified toxic pollutants to be monitored are the 129 priority pollutants plus six pesticides listed in Table 3.

The primary method to be considered for determining levels of toxic substance bioaccumulation in the vicinity of the outfall should be through the use of caged specimens of bivalve molluscs. Recommended methods are provided by EPA (1982). In situ biomonitoring has been used to monitor levels of toxic substances in the water column (Young et al., 1976). Generally, mussels (Mytilus californianus or M. edulis) or oysters (Crassostrea spp.) should be utilized as the test organisms. These species are widely distributed and easily collected in large numbers in most coastal areas of the U.S. Also, there is a considerable amount of literature concerning the rate of uptake of specific substances, experimental survival, and the selective uptake of the various groups of toxic substances by different tissues (see, for example: de Lappe et al. 1972; Young and Heesen 1974; Clark and Finley 1973; Alexander and Young 1976; and Eganhouse and Young 1976). Other filter-feeding molluscs have been used in a similar manner to monitor toxic substances in the marine environment (Goldberg et al., 1978), and these organisms could be substituted if conditions are not appropriate for survival of mussels or oysters.

Although minimum numbers of replicate samples and specimens are specified in EPA (1982), the investigation of site-specific environmental characteristics, seasonal variability in background pollution concentration levels, and, most importantly, variability in the uptake of different toxic pollutants should be examined during the initial stages of the bioaccumulation monitoring program. The objective of these preliminary investigations should be to determine the number of replicate samples and number of organisms included in composite samples which will result in the optimal sampling program, i.e., a program that will provide the basic assessment information at a minimum cost for sample collection and analysis.

The monitoring of toxic pollutant concentrations in tissue samples from resident macroinvertebrates and fish species should be conducted in cases where the bioaccumulation of toxic pollutants has been documented or the potential for accumulation in the food chain is considered to be high. Emphasis should be placed on the selection of commercially or recreationally important species for which information is available on the uptake and effects of elevated toxic pollutant concentrations. Composite samples consisting of at least six specimens should be collected at the specified stations and sampling periods for tissue analysis. Since the objectives of this phase of the program are to investigate accumulation of toxic pollutants in the food chain and to assess the suitability of commercially or recreationally important species for human consumption, emphasis should be placed on the determination of toxic pollutant concentrations in muscle tissue.

At the time of sample collection, the length, weight, and mortality (in the case of the caged bivalves) should be recorded. The physiological condition of all organsims (e.g., the presence of external lesions and discoloration) should also be noted. The observed concentrations of identified toxic pollutants in tissue samples should be reported in both tabular and graphic form. Statistical comparisons of the observed concentrations of toxic substances in tissue samples should be made to determine the existence of significant differences among stations or replicates. Degree of fouling of cages and presence of potential predators (e.g., crabs) within cages should be noted.

Fishes--

Marine fish communities are complex and dynamic in nature. The structure of these communities changes seasonally as a result of spawning, migrations, and recruitment of juvenile fish to adult populations. In the short term, feeding activities (including diel movements) will influence observed community composition. Selective characteristics of various types of fishing gear tend to confound this problem (Hamley 1975) since, for example, different sized (and aged) individuals from the same species will be selected differently by different gear types, while individuals of the same size from different species will also exhibit differences in catchability. Catchability also varies on a diel basis as a result of changes in avoidance capabilities under different light conditions, effects of tidal currents on activity patterns, and other factors.

Extreme spatial heterogeneity is a characteristic aspect of the distribution of many species of demersal and (especially) pelagic species; sampling plans which fail to take spatial heterogeneity into consideration can result in biased conclusions.

Spatial and temporal variation of this kind places significant demands on the design of sampling surveys. For example, Richkus (1980) reports that, in a study conducted by Texas Instruments, Inc., in Chesapeake Bay, it was determined that it would be necessary to collect 252 samples to produce an 80 percent chance of detecting a 50 percent difference in density of white perch (Morone americana) among three locations. Thus, the objectives of a monitoring program for fishes, together with the structure of the target community, will exert a major influence on many aspects of the design of that program.

Several different types of gear have been used for sampling fish communities. The selection of gear which is appropriate to address specific survey objectives will depend upon the substrate type, the communities to be sampled, tidal and other current conditions, depth, proximity to the shore, and survey vessel capabilities (Table 7). A discussion of the applicability of various fish sampling techniques is included in Richkus (1980). Von Brandt (1972) provides a general review of fish catching methods. Uzmann et al. (1977) present a comparison of three survey techniques.

When demersal fish populations are to be sampled in areas of sand or mud bottom, use of an otter trawl is appropriate. The Marinovich 7.62-m (25-ft) headrope otter trawl, described as the Coastal Water Project Marinovich net in Table 3 of Mearns and Stubbs (1974), is recommended for this purpose. It is commonly used for environmental survey work and is easily handled from a small boat (Mearns and Stubbs 1974). The net is towed from a single warp. Gear specifications and sampling procedures are critically important. A discussion is provided by Mearns and Allen (1978), but several additional points are important:

- Steel (or stainless steel) towing cable of 6.35 mm (0.25 in) minimum diameter is recommended.

- A power winch is required; gear must be recovered while the vessel is moving forward.

- Effort must be reported in terms of distance or area covered. Fixed buoys or navigational aids (e.g., the Mini Ranger) will be useful in this context. Haul distances of

TABLE 7. SELECTION OF APPROPRIATE FISH SAMPLING GEAR

Habitat	Primary Approach	Secondary Approach
Demersal	Otter Trawl (Gillnet, Trammel Net, or Trap)[a]	Diving Submersible Hook and Line
Pelagic	Commercial Monitoring, Gillnet	Acoustic Transect Pelagic Trawl Lampara/Purse Seine
Nearshore	Beach Seine	

[a] If not trawlable.

700 - 1,000 m (2,297 to 3,281 ft) are recommended. Information on vessel speed and haul duration should be recorded but cannot substitute for distance estimates.

- A towing speed (relative to the bottom) of 1.3 m/sec (2.5 knots) is recommended.
- The gear should be towed into the current and along an isobath. Current conditions should be recorded.

A fundamental problem of demersal trawl sampling concerns the manner with which this gear samples pelagic forms. These species (or life history stages) often exhibit schooling behavior. Incidental encounter of pelagic forms occurs during setting and recovery of gear; this is inconsistent, but individual species behavior is not always understood well enough to permit objective exclusion of these data. Species that are unquestionably pelagic in habit (such as the northern anchovy Engraulis mordax) should be excluded from demersal trawl catch data. This problem can also be addressed by separately analyzing data for one segment of the fish community which is known to be demersal (such as the flatfishes). An awareness of the selective characteristics of trawl gear and the behavior of the gear itself is also important (Wathne 1977, Harden Jones et al. 1977).

Gillnets and trammel nets are often utilized in areas where bottom conditions preclude trawling or where improved spatial resolution is required (such as within the ZID and at comparable stations). Variable mesh, set gillnets are recommended (Ricker 1971), although trammel nets (Becker et al., 1975) may be appropriate in some situations. Traps also provide high spatial resolution (Becker et al., 1975) but are highly selective.

In situations where nearshore fishes need to be sampled (such as when an outfall is located in an area of juvenile salmonid migration), beach seine sampling should be conducted (Allen et al., 1959).

Pelagic forms are often ignored during survey sampling. Pelagic species are, however, important in many of the areas for which 301(h) applications have been received. In a situation where the species of concern are subject to consistent commercial or recreational exploitation in the vicinity of the outfall, fisheries surveys (see below) can be utilized to collect appropriate data. When this is not possible, the use of acoustic (and sonar) transects and pelagic sampling nets (such as pelagic trawls, lamparas, and purse seines) is recommended (Saville 1977; Becker et al., 1975; Lemberg 1978; Fiedler 1978; Richkus 1980).

Specific data can be collected from the immediate vicinity of the outfall structures by means of diving and submersible surveys. Details are provided by Allen et al. (1976), Allen (1975), and Becker et al. (1975). Line transect techniques often provide an appropriate method for quantifying diving observations; quantitative procedures are discussed by Seber (1973). Some field methods are presented by Fager et al. (1966) and Walton and Bartoo (1976).

Hook-and-line surveys are especially useful for sampling in precise locations and for obtaining larger individuals which may avoid other sampling gear. This type of gear can be used in areas which may not be accessible to other sampling devices and is frequently employed to provide specimens for bioaccumulation analysis. Allen et al. (1975) describe appropriate techniques. It should be noted that hook-and-line techniques are especially selective in nature; hook size, bait type, and other gear specifications should be selected with this consideration in mind.

Data collection requirements will depend on specific survey objectives. At a minimum, all fish catches should be identified to species, and counted and weighed by species. Taxonomic procedures and authorities should be clearly defined and a procedure for seeking expert advice should be included, if specimens cannot be identified by employees or consultants.

For individual species, length-frequency and length-weight analyses may be required to allow consideration of population differences between outfall and reference sites. Standard length is the recommended measurement; if a different length measurement is recorded, this should be stated, and a regression relationship between this measurement and standard length should be provided together with the data utilized in the analysis. Appropriate subsampling procedures must be defined for the collection of these data. When individual observations are recorded, life history stage should also be reported. Unless subsampling is to be conducted, all individuals in the catches should be examined for external disease symptoms or abnormalities; a standardized technique for identifying and defining abnormalities should be developed and included in the data reports. Each individual observation should be recorded; the use of computer data coding forms is recommended for this purpose. All raw data observations, identified by sample number, station location, date and time of collection, and individuals responsible, should be included as an appendix to reports.

Commercial and Recreational Fisheries--

If commercial or recreational fisheries activities are conducted in the vicinity of an outfall, these activities must be monitored. Commercial and recreational fisheries catch data are generally reported as summary statistics for statistical blocks defined by the state agencies concerned. In most cases, the area covered by these statistical blocks is too large to allow resolution of fishery catch conditions close to a sewage outfall.

Coordination with state agencies may provide an effective and inexpensive mechanism for collecting data which can be used to assess the condition and productivity of specific fisheries. A voluntary logbook program for fishermen could be designed which would allow those fishing in areas of concern to record data on catch and effort close to the outfall and at remote locations. In some cases, vessels observed to be fishing close to an outfall could be identified and the operators interviewed when the vessels dock. Similarly, individuals observed sportfishing could be identified and interviewed.

An alternative approach to monitoring recreational fishing activities would involve sampling at the outfall and reference area with appropriate sportfishing gear. This would allow direct comparison of species caught, catch rates, disease prevalence, bioaccumulation, and other relevant aspects.

Market or consumer acceptability of fish caught in the vicinity of a POTW outfall should also be addressed in all commercial and recreational fisheries surveys; a simple interview procedure would be appropriate.

Recreational harvesting of intertidal shellfish resources occurs in the vicinity of some POTW outfalls. These shellfish should be sampled with respect to both public health considerations and possible population responses to discharge effects. While quadrat sampling (Seber 1973) is a theoretically attractive approach to this problem, the time and effort involved would be prohibitive in most cases. Monitoring of recreational catch and effort (in association with the appropriate government agency) is an appropriate way to examine relative population abundances.

When interviews, voluntary logbooks, and field observations of fishing activity are utilized for data collection, a complete log of all relevant information should be maintained by the POTW. Interviews should be conducted by means of questionnaire. The log should contain all interview and logbook returns and detailed records of field observations; details of public health analysis of shellfish will also be included. All entries should be identified by time, date, and the individuals who collected the information. A clear and comprehensive copy of this log should be included as an appendix to monitoring reports.

Phytoplankton--

Since phytoplankton are transient, a monitoring program to sample phytoplankton should be designed somewhat differently from monitoring programs for certain other biotic groups. Phytoplankton are carried about by movements of the water, and consequently maximum sewage effluent impacts on phytoplankton may occur well away from an outfall. Stations should be located at sufficient distance from an outfall to accommodate a lag time in the response of phytoplankton to sewage effluent inputs. Due to their short turnover times (on the order of hours to days), phytoplankton communities may respond to perturbations much more rapidly than other biotic groups; therefore, samples must be collected more frequently. Bimonthly samples are probably the least frequent which could be expected to give meaningful results, although monthly or even biweekly samples would be preferable.

In situations where phytoplankton communities display pronounced seasonal variations in standing stock or production, it may be appropriate to use a temporally stratified sampling approach. For example, if phytoplankton growth is highest during the spring, sampling may be conducted on a frequent basis (e.g., weekly). Similarly, during periods of consistently low phytoplankton growth, a reduced sampling frequency may be used.

Adequate assessment of phytoplankton community response to sewage discharges will generally involve more sampling stations than would be required for the benthos. Thus, it is important to initially assess whether or not a phytoplankton sampling program is justified based on a consideration of discharge size, sensitivity of receiving water, and evidence of previous impacts on phytoplankton. Selection of phytoplankton sampling stations should always involve a thorough consideration of water circulation patterns to ensure that putative waste field stations are actually being exposed to the diluted effluent and that control stations are not subject to influence of the waste field. If evaluation of circulation patterns indicates that the sewage waste field may be transported to areas of limited flushing (e.g., embayments or eddies), special emphasis should be placed upon locating sampling stations in these areas. In all cases, phytoplankton sampling stations should be located in areas of maximum predicted effects, considering such factors as response lag time, effluent dilution, and circulation patterns.

The most likely direct effect of sewage effluent on phytoplankton communities is enhancement or inhibition of primary production. Enhancement may occur in areas where the phytoplankton are naturally nutrient limited, since sewage effluent represents a significant source of nutrients. Inhibition may occur if there are sufficient concentrations of toxic or inhibitory substances in the effluent.

In areas where phytoplankton production is enhanced (or inhibited), the standing stock of phytoplankton may be expected to be higher (or lower) than in reference areas. Measurement of the concentration of chlorophyll _a_ in the water is an indirect method of analyzing the standing stock of phytoplankton. It is recommended that the two-dimensional spatial distribution of chlorophyll _a_ concentrations about the outfall be analyzed. Samples should be collected at several distances from the outfall in the direction of current flow. Samples should also be collected at a variety of depths throughout the euphotic zone (from the surface to the 1-percent light level, as estimated from any light transmittance data collected as part of the Water Quality Monitoring Program).

The vertical distribution of chlorophyll a concentrations at each station may be examined through the collection of water samples with water bottles at various depths (followed by fluorometric or spectrophotometric determination of chlorophyll a) or, if available, a pump system may be used with a flow-through fluorometer (Lorenzen 1966) for a continuous profile of chlorophyll a concentrations vs. depth. The advantages and disadvantages of various water bottles are discussed by Venrick (1978a), while the use of pumps is discussed by Beers (1978). It is advisable that a pump be used only for the determination of chlorophyll a and that water bottles be used for the collection of phytoplankton for productivity measurements and taxonomic analyses, since the inevitable agitation associated with pumping may damage some cells.

If it is determined that the vertical distribution of phytoplankton biomass (as mg chlorophyll a/m^3) is reasonably uniform throughout the euphotic zone, water samples for simulated in situ primary productivity measurements (Ahlstrom 1969) may be collected with water bottles at depths corresponding to fixed percentages of incident solar radiation. If, however, there is significant vertical stratification of the phytoplankton community, sampling depths should be adjusted so that samples are also collected within subsurface chlorophyll maxima or minima. Phytoplankton primary productivity should be measured by the ^{14}C light-dark bottle technique as described by UNESCO (1973), and measurements at each station-depth should be replicated to facilitate statistical analysis.

If the monitoring program described above reveals perturbations of chlorophyll a concentrations and/or primary productivity within or beyond the ZID, taxonomic analyses should be conducted since phytoplankton species vary in their responses to alterations in their nutrient source (Eppley et al., 1969) or in their responses to certain inhibitory substances (Thomas and Seibert 1977). Subsamples should be drawn from the water collected in the water sampling bottles and preserved for later microscopic analysis onshore. It is important that samples for taxonomic analysis be collected at various depths throughout the euphotic zone since different species may have different depth distributions. It is also important that sampling be conducted at similar times during the day (i.e., mid-morning or mid-afternoon) since some phytoplankton are known to migrate vertically (Stofan and Grant 1978).

Choice of a fixative depends somewhat on the dominant types of phytoplankton known to inhabit a given area. Buffered formaldehyde and Lugol's solution are two common fixatives. The advantages and disadvantages of each are discussed by Throndsen (1978a).

Taxonomic analysis almost always involves some form of subsampling, which is consequently a potential source of bias or variability. The statistical implications of subsampling are discussed by Venrick (1978b). Preserved phytoplankton samples normally must be concentrated for quantitative microscopic analysis. Although other methods are available (Sukhanova 1978, Throndsen 1978b), the routine method is the Utermohl technique, which utilizes sedimentation cylinders and an inverted microscope (Hasle 1978).

Taxonomic analysis should include identification of the dominant phytoplankton taxa and counts of individual species whenever possible. Numerous taxonomic references are available [see Chapter 6.4 of UNESCO (1978)]. The accuracy and consistency of phytoplankton identifications are of the utmost importance for characterization of the BIP, both in the reference area and at stations in the vicinity of the discharge. Counts of individual species should be standardized to numbers per unit volume of the original water-bottle sample, calculated with consideration for whatever subsampling technique was utilized.

If replicate taxonomic samples are available for each station-depth, the estimates of abundance of individual species may be analyzed statistically for differences among depths, among stations, or among times. Particular attention should be given to differences in community composition between stations in the vicinity of the outfall and stations in a reference area. Species diversity, richness (number of species), evenness, or numerous other parameters (Pielou 1970) may be utilized for description and comparison of the phytoplankton communities.

If available information indicates a potential for enhancement of individual phytoplankton groups (especially dinoflagellates), the monitoring program should include an assessment of the magnitude, duration, and point of initiation for phytoplankton blooms. Special emphasis should be placed upon species causing accumulation of toxins in other organisms, or blooms which may result in fish kills.

The goal of the phytoplankton monitoring program should be to demonstrate whether or not the discharge of sewage effluent from the outfall in question interferes with the protection and propagation or the natural range of variation of phytoplankton in areas beyond the ZID. Characteristics of phytoplankton which may be examined include the community biomass (as estimated through the measurement of chlorophyll _a_ concentrations), community primary productivity (as estimated through simulated in situ incubations using the light-dark bottle technique), and the various community composition parameters. The responses of biological communities to pollutant stress appear to involve a continuum, as indicated by the gradients in the biological variables of the benthos near sources of organic pollutants (Pearson and Rosenberg 1978). Therefore, alteration to the phytoplankton communities should be analyzed in relation to potential or determined (by other community studies) impacts on other biological communities which make up a balanced indigenous population. This analysis should include, although it should not be limited to, food web impacts, the occurrence of toxic or nuisance phytoplankton, eutrophication or blooms, and potential second impacts on zooplankton or fish communities.

Zooplankton--

Zooplankton, like phytoplankton, are transient, and, consequently, a monitoring program designed to sample zooplankton should be designed somewhat differently from monitoring programs for certain other biotic groups that tend to be permanent residents of an area. Zooplankton are carried about by movements of the water; therefore, the maximum sewage effluent impacts on zooplankton may occur at some distance away from the outfall. Unlike phytoplankton, however, zooplankton life spans are typically on the order of a few months, so their capacity for responding to perturbations is much less than that of phytoplankton. Bimonthly samples are usually adequate for analysis of changes in zooplankton communities. Zooplankton possess varying degrees of swimming ability and therefore have the potential for aggregating in patches or in narrow depth strata, which introduces an additional complication in quantitative sampling. In addition, the ability to swim means that many zooplankton can avoid certain types of sampling gear.

As is the case for phytoplankton, the design of zooplankton sampling programs should consider natural temporal fluctuations in abundance and species composition. Zooplankton assemblages display a high degree of spatial heterogeneity in addition to pronounced diel vertical migrations by many groups. These factors, combined with a longer response time to effects of sewage effluents, would result in the necessity of conducting relatively extensive programs (i.e., in number of sampling stations, frequency of sampling, and replications) to adequately assess responses of zooplankton to pollutant inputs. Thus, studies of zooplankton assemblages should be conducted only when there is evidence of previous impact in zooplankton, when phytoplankton communities display significant effects, or when large discharges are located in areas where there is a high potential impact on zooplankton (e.g., in estuarine environments with important macroplanktonic larvae of commercial and recreational species).

For zooplankton, there is no easily measured functional response to POTW effluent discharges similar to primary productivity for phytoplankton. Toxic effects of effluent on zooplankton are possible if there are sufficient concentrations of toxic substances in the effluent. Alteration of zooplankton community composition is a distinct possibility in areas where the phytoplankton community composition has been affected, since many zooplankton graze on phytoplankton. Given the smaller proportion of their life spans spent within the sphere of influence of the outfall, zooplankton are less likely to experience direct, observable changes in community composition than are phytoplankton.

The zooplankton encompasses a wide range of organisms, from microscopic protozoans to large planktonic crustaceans and larval fish. In general, the smaller organisms have shorter life spans, so effluent impacts are more likely among the smaller organisms. Sampling methods vary depending upon the size of the organisms.

Microzooplankton (those which pass through the mesh of a 202-um net) can be collected either with water bottles similar to those used for phytoplankton collection [although a volume of at least 10 liters is recommended (Jacobs and Grant 1978)] or with pumping systems (cf., Beers et al., 1967). If water bottles are used, samples should be collected from a variety of depths throughout the water column, and the captured organisms may be concentrated with a fine-mesh (e.g., 63-um) screen. Replicate samples should be collected from each station-depth. Pumping systems may incorporate a single filter or a mesh of different size filters for collection of the organisms. Flow rate should be in the range of 150 - 200 l/min (Jacobs and Grant 1978). Pumping systems have the advantage of being able to take samples integrated over depth, or of collecting samples while the ship is underway, but they may damage soft-bodied organisms, and they are more expensive and complicated than water bottles.

For collection of small mesozooplankton (those retained on a 202-um mesh), nets are generally used. For an excellent discussion of net design and function, see UNESCO (1968). Nets with small mouth diameters (20 - 40 cm) may introduce error by underestimating abundance and diversity (McGowan and Fraundorf 1966, Wiebe and Holland 1968). A minimum mouth diameter of 60 cm is generally recommended (Jacobs and Grant 1978). With mesh sizes of less than 202 um, clogging and loss of filtration efficiency are often a problem, so a 202-um mesh is the smallest which should be used (UNESCO 1968). Additional tows may have to be made with larger nets (1.0-m mouth diameter and 505-um mesh) in order to collect representative samples of larger zooplankton and larval fish. All tows should be replicated; the number of replicates necessary for the desired precision of estimates should be determined during a preliminary or pilot sampling program (cf., Cochran 1963; Green 1979). Paired bongo nets (McGowan and Brown 1966) are often used because they provide two replicate samples from the same environment. Alternatively, they can be rigged with two different mesh nets for collection of different size fractions. Any net used should have a flow meter attached to the mouth for calculation of volume filtered. Prior to selection of a sampling technique, the spatial and temporal distributional characteristics of the target zooplankton assemblage should be considered. For example, lobster (Homarus americanus) larvae occur near the water surface and are appropriately sampled by a neuston net.

Oblique tows are highly recommended, with sampling extending from just above the bottom to the surface. Avoidance by larger zooplankton is significant at slow tow speeds, so a ship's speed of 1.5 - 2 knots should be maintained (Jacobs and Grant 1978). The animals collected should be washed (from the outside of the net) into the cod end, transferred to a labeled sample jar, and preserved with buffered formalin in the proportion of nine parts sample to one part formalin (a saturated solution containing 38- to 40-percent formaldehyde). Further discussion of shipboard handling of samples can be found in Jacobs and Grant (1978).

For quantitative taxonomic analysis of the zooplankton samples, subsampling will normally be required. Two methods which are commonly used are: subsampling by Stempel pipette, and splitting with either a Folsom splitter (McEwen et al., 1954) or the newer Burrell et al. (1974) device. In either case, large and/or rare organisms should first be counted and removed. The use of the Stempel pipette is discussed by Jacobs and Grant (1978), who point out that due to the small aliquot size, this method should only be used when rapid "ballpark" numbers are needed. The use of plankton sample splitters is also discussed by Jacobs and Grant (1978), and they indicate that this is the best method for quantitative analysis of zooplankton. The counts of individual taxa should be transformed to numbers per sample (considering the subsample size) and then standarized to numbers per unit volume of water filtered (calculated from the flowmeter reading).

Taxonomic analysis of the samples should include identification of the dominant zooplankton taxa and counts of individual species whenever possible. Particular attention should be given to the meroplanktonic larvae of commercially and ecologically important species (e.g., fish, shrimp, lobsters, etc.). The accuracy and consistency of zooplankton identifications are of the utmost importance for characterization of the range of variation of the zooplankton communities, both in the reference area and at the various outfall stations.

If replicate taxonomic samples are available for each station, the estimates of abundance of individual species may be analyzed statistically for differences among stations or among times. Particular attention should be given to differences in community composition between stations in the vicinity of the outfall and stations in a reference area. Species diversity, richness (number of species), evenness, or numerous other parameters (Pielou 1970) may be utilized for description and comparison of the zooplankton communities.

The goal of the zooplankton monitoring program should be to demonstrate whether or not the discharge of sewage effluent from the outfall in question interferes with the protection and propagation or the natural range of variation of the zooplankton communities in areas beyond the ZID. Characteristics of the zooplankton communities should include, but not necessarily be limited to, species composition, abundance, dominance, and diversity. The responses of biological communities to pollutant stress appear to involve a continuum, as indicated by the gradients in biological variables of the benthos near sources of organic pollutants (Pearson and Rosenberg 1978). Therefore, alteration in the zooplankton communities should be analyzed in relation to potential or determined (by other community studies) impacts on other biiological communities which would make up a balanced indigenous population. This analysis should include, although it should not be limited to, the structure and function of both larval and adult zooplankton communities, as well as consideration of food web impacts.

Kelp Communities--

Kelp beds are distinctive habitats of limited distribution whose protection is of special concern because of their ecological significance and their economic value to man. The kelp plants themselves are largely responsible for the spatial structure of this community, as they provide food, substrate, and shelter for a variety of organisms (Tegner 1980). In some areas, the kelp itself is harvested, and in many areas the kelp beds are the location of valuable fisheries for abalone, lobster, fishes, and sea urchins (Tegner 1980). Kelp beds may be particularly sensitive to outfall discharges, and adverse effects of sewage effluent on kelp have been suggested by Carlisle (1968), Mearns et al. (1977), and others. If kelp bed communities are potentially affected by a sewage effluent discharge, a monitoring program should be conducted to evaluate the health and extent of these communities.

Kelp bed communities typically include a great variety of plant and animal species, but since the continued existence of the community is largely dependent on the presence of the kelp plants themselves, the monitoring program should focus on the health and spatial distribution of these plants, rather than attempt a detailed analysis of the entire community. The location, condition, and size of kelp beds along the southern California coast have been monitored for a number of years through the use of aerial surveys (Wilson et al., 1980). This is a particularly useful technique because changes in the areal extent of the beds can be monitored over time, and areas of potential sewage impact can be identified.

Aerial surveys of kelp beds utilize infrared photographs taken from an aircraft flying at an altitude of 1.5 - 2.6 km (0.9 - 1.6 mi). Photographs taken around midday will minimize reflected glare, and a polarizing filter may be used, if necessary. Overlapping adjacent photographs (10 - 20 percent) assure full coverage and minimize barrel distortion at the film edges. Slides of the kelp beds can be projected and drawn onto charts of the coast, and the surface area of the kelp canopies can be calculated from these charts using a polar planimeter or a measured grid network (Wilson et al., 1980).

If a decline in nearby kelp beds occurs, the discharge of sewage effluent may or may not be the cause or a cause. Sewage effluent may adversely impact kelp in a variety of ways. Certain constituents of the effluent may be toxic to kelp sporophytes and/or gametophytes, and the plants may die in areas where destructive concentrations of these constituents appear. Turbidity within the discharge field of the outfall might be increased, reducing light intensities or altering the spectral distribution of the light such that kelp growth is adversely affected. Concentrations of kelp enemies such as grazers, pathogens, or parasites might in some way be enhanced by the effluent, and the increase in their numbers might bring about a decline in the kelp. Finally, siltation effects from the settling of suspended matter discharged by the outfall might interfere with the recruitment of young kelp plants (North 1964).

There have been suggestions (Wilson et al., 1980) that sewage effluent discharge may have decreased the maximum depth of kelp growth in nearby kelp beds. If the light transmittance data collected as part of the water quality monitoring program is also collected on a regular basis along the seaward edge of nearby kelp beds, it should be possible, given the known photosynthetic requirements of kelp (Clendenning 1964; Rosenthal et al., 1974), to determine whether the sewage effluent discharge may be adversely impacting the kelp beds. Comparison of light transmittance measurements at specific depths along the kelp bed in question with those at similar depths near other kelp beds removed from anthropogenic sources of sedimentary material should indicate whether the kelp bed in question could extend into deeper water in the absence of the effluent discharge.

The use of sediment traps to quantify the amount of sedimentation occurring along the margins of potentially impacted kelp beds should also be considered. Comparison of the rates of sedimentation there with those along kelp beds removed from anthropogenic sources of sediments, and consideration of the effects of different amounts of sediment on the survival and growth of kelp germling stages (Devinny and Volse 1978), may permit an evaluation of whether or not the sewage effluent discharge may be inhibiting expansion of the kelp bed in question.

While the toxicity of certain effluent constituents on kelp has been studied (North 1964), it is probably unreasonable to expect that detailed studies of toxic effects would be conducted as part of a kelp monitoring program, given the large number of potentially toxic or inhibitory substances in most municipal effluents and the possibility for complicated synergistic effects.

Increased abundances of certain animals which graze on kelp [notably sea urchins (see Lawrence 1975)], have been implicated in the decline of certain kelp beds. While there have been suggestions that the abundances of these grazers may be enhanced by the discharge of sewage effluent (Clark 1969), it is difficult to establish cause-and-effect relationships. The true cause of the increased abundances of these organisms may only be revealed through detailed investigations of interspecific interactions and predator-prey relationships (Tegner 1980), which may be beyond the scope of individual 301(h) monitoring programs.

One promising method which may be used to infer causes of observed changes in kelp canopy size is regression analysis, utilizing such factors as suspended solids, mass emission rates, water temperature, water transparency, etc. Wilson et al. (1980) used this method to examine potential causes of the initial decline and subsequent recovery of kelp beds along the Palos Verdes Peninsula, in close proximity to a very large sewage outfall. Nevertheless, identifying changes which have occurred in kelp canopy size is somewhat easier than deciding what factor(s) may be responsible.

Coral Communities--

In some areas, it will be important to assess any impact the applicant's discharge may have on coral communities. Generally, an assessment of changes on living coral coverage which may include a study of reef fishes, will be sufficient rather than a complete study of the reef community.

A line transect method of sampling should be used for studies of both the living coral coverage and reef fishes. All stations should be comparable as far as the distance from sand areas, rubble, and base-rock relief. At each station, a 50-m (164.1-ft) length of electrical wire should be permanently attached to the reef, parallel to the shoreline. Care should be taken to ensure that the line is located within a reef area of sufficient size so as to eliminate any patchiness in the data due to sand area or reef edge effects.

Photographs of at least 0.67 m^2 (7.24 ft^2) of the bottom should be taken on the shoreward side of the line at 5-m (16.4-ft) intervals. An underwater camera mounted on a rigid framing device should be used. Each photograph should contain a small slate indicating the station, date, and position of the photograph along the line. Care shall be taken in order to be certain of photographing the same quadrat each quarter. It is suggested that stakes be driven or cemented to the reef indicating at least two corners of each frame.

The photographs should be developed as slides. These slides should be projected onto a grid having the dimensions of the original quadrat, and the percent coverage of coral and encrusting algae by species, and of the noncoral substrate, should be estimated.

Other transecting methods may be used to sample living coral coverage if they are shown to be statistically valid sampling techniques; however, due to the relative ease of sampling and data reduction, the photographic method described above is recommended.

The investigation of reef fishes should consist of SCUBA transects along the transect lines used for the reef benthos survey. Beginning at least 1 day after the lines are permanently attached to the reef, a diver should swim each line in order to identify and count the fishes located within 3 m (9.8 ft) either side of the line. The diver should take care to enter the water away from the transect area in order to avoid disturbing the fishes. One transect at each station should be completed during each of three consecutive days for each quarter.

Consistency of technique is important; therefore, the applicant should make every effort to ensure that transects are conducted in a similar manner by the same diver-biologist if possible. Some investigators are known to look for cryptic species more than others, or to notice larger fish high in the water column more readily. Such sources of variability should be kept to a minimum.

Where applicable, those quality assurance/quality control (QA/QC) procedures specified for the benthos should be followed for the coral and fishes. These procedures include the maintenance of a voucher collection, verification of identifications, inclusion of raw data sheets in reports, etc. In addition, it is important that the applicant maintain consistency between sampling periods in order to reduce sampling variability.

The monitoring report should contain a tabulation of the percentage of living coral, coralline algae, and coral rubble for each photographic quadrat. A tabulation of the number of individuals of each species of fish identified on each transect should also be included.

A field notebook, as discussed in the benthos section above, should be maintained and submitted along with the monitoring report.

Intertidal Communities--

Due to the offshore location of most marine sewage outfalls, monitoring of intertidal communities will usually not be specified as part of the biological monitoring program. Nevertheless, intertidal communities are sensitive assemblages of organisms which may be affected by sewage discharges (Dawson 1959, 1965). In cases where shoreward transport of the waste field is predicted, monitoring the intertidal community may be required.

The type of intertidal community is largely determined by the type of substrate (tidal flats, sandy beaches, rocky shores, gravel and cobble shores), and the appropriate sampling procedures vary considerably among the various communities represented in these environments. Gonor and Kemp (1978) provide a comprehensive review of the procedures used in quantitative ecological assessments in the various types of intertidal environments; it is unnecessary to provide a detailed reiteration of the sampling procedures described therein for each habitat and biotic group. Selected sampling procedures for rocky intertidal habitats will instead be described in order to illustrate some of the basic principles involved.

A common attribute of many intertidal communities is the stratification of the community with respect to tidal height, since many intertidal species have discrete vertical limits within the tidal range. Since both community species composition and density vary considerably with slight changes of vertical distance (on the order of tenths of a meter), a sampling plan designed to monitor the entire intertidal community should be stratified by vertical height. Comparison of communities between reference areas and discharge impact areas should be between samples from similar elevation, also. If data for an entire transect across the intertidal community are pooled, it is unlikely that differences between reference and discharge impact areas could be detected because the within-transect variance produced by combining data from different levels would be enormous. For an example of the application of these principles, see Batzli (1969).

In order to detect differences between areas, or changes at one location through time which arise from anthropogenic perturbations, these differences or changes should be distinguished from the natural spatial and temporal variations at a given location. Field sampling should, therefore, quantify the spatial and temporal heterogeneity of each site. Intertidal sampling is typically conducted along a transect. Samples are usually collected (or a census of the community is conducted) at various locations along the transect. These locations may be spaced evenly along the transect (systematic sampling) or randomly along the transect (random sampling). The advantages and disadvantages of each are discussed by Cochran (1977).

Considerations for the selection of the number of transects, the length and width of the transects, and the number and size of the sampling units are discussed by Gonor and Kemp (1978). Samples are normally collected (or the census of the community is conducted) within quadrats along the transects (i.e., plots of constant area). The size of the quadrat is a function of the nature of the species to be examined, their relative abundance, and the cost of collecting (or conducting a census of) the organisms. Gonor and Kemp (1978) recommend that a preliminary sampling program be conducted in order to investigate the variability of a given area and to determine the relative efficiency of various quadrat sizes and numbers of samples.

Sampling intertidal communities can be either destructive (in which a quantitative sample of the biota is removed for later analysis), or nondestructive (the acquisition of similar data using methods which do not disturb the communty). Aside from a desire to minimize the damage done to an area, there exists a second reason for favoring nondestructive sampling, i.e., that repeated sampling of an area could be biased by the effects of previous sampling. It is known, for instance, that reduction in the abundance of certain keystone species (Paine 1969) may alter the rest of the community in such a manner that change due to other events may be undetectable.

Nondestructive sampling can generally be used if the species to be sampled are visible, measurable, and unaffected by the sampling procedure used (Gonor and Kemp 1978). Both the macroalgae and the sessile macrofauna on rocky shores may be sampled without removal. Individuals within a quadrat can be counted and measured in situ for some suitable dimension (e.g., percent cover) which can be converted into an estimate of biomass (Gonor and Kemp 1978). Nondestructive sampling is inadequate for both small and mobile species, however, so if the entire community is to be censused, some destructive sampling must occur. For most impact studies, however, it may be sufficient to only examine effects on the plants and animals which can be sampled nondestructively. Nondestructive sampling in the field may be supplemented with photography (Littler 1971), which is particularly useful when time in the field is severely limited.

Littler and Murray (1975) investigated the biological effects of a low-volume domestic sewage discharge on the intertidal community on San

Clemente Island, using sampling techniques which illustrate well the sampling design considerations described above. Their study could be used as a model for monitoring programs to be conducted in other intertidal communities.

Littler and Murray (1975) determined the distributions and abundances of macro-organisms with reference to tidal height and distance from the outfall. They utilized a photographimetric technique (Littler 1971) for assessing standing stocks (i.e., frequency and cover) of species populations. Sampling was restricted to macro-epibiota which could be discerned with the unaided eye in the field or in photographs. Photographs were taken of ring quadrats 30 cm in diameter (providing 0.07-m^2 stratified plots) at 1- or 2-m intervals along transects both in the outfall area and at randomly selected points in control areas. The control areas were sufficiently remote from the influence of the outfall and had morphometry similar to the area in the immediate vicinity of the discharge.

Cover was determined (Littler and Murray 1975) from the photographs using a point-intercept method. If species were observed within a quadrat but were absent from the scores, they were assigned a cover value of 0.05 percent. In some cases, samples contained multi-layered algal canopies; thus, total cover was in excess of 100 percent. In these cases, more than one photograph had to be taken per quadrat to measure stratification. Field notes were taken using a tape recorder, which then facilitated later taxonomic analysis of the samples.

Vertical heights for each quadrat were measured from fixed reference points using a sighting level, a stadia rod, and standard surveying techniques. Relative tidal heights were referenced to the level of mean-lower-low-water (MLLW).

Sampling in different seasons (Littler and Murray 1975) showed that seasonal changes in standing stock were minor, especially in the sewage-affected area, so data for all sampling periods were considered to be representative and were grouped as either outfall or control samples. If seasonal changes had been significant, comparisons of outfall and control samples would probably have had to be restricted to within a given season.

The intertidal communities were stratified by 0.15-m intervals of tidal height. The distribution of species populations as a function of tidal height was then compared between the outfall and control areas. The community features of species diversity, stratification, and species assemblages were analyzed using the cover data. Cluster analysis was utilized to objectively determine natural assemblages or groupings of organisms.

The techniques utilized by Littler and Murray (1975) could easily be applied to monitoring programs in other rocky intertidal environments. While certain of the principles involved may apply to other environments, sampling techniques will probably differ, and the more complete discussion of Gonor and Kemp (1978) should be consulted.

Analytical Techniques

Introduction--

The evaluation of compliance with BIP maintenance requirements necessitates the analysis of biological monitoring data including comparisons of spatial and temporal variability in the composition and structure of the benthic macrofaunal assemblages and other selected biotic groups among monitoring stations. A wide variety of techniques are available for such analysis of biological data collected as part of a 301(h) monitoring program. Analyses may range from rather simple qualitative (tabular or graphical) comparisons of species distributions to complex multivariate tests of the relationships of community structure to environmental variables. The following sections provide discussions of the applicability of several analytical techniques; however, no single approach is recommended for analyzing the monitoring program data. In most cases, the optimal approach will be to utilize several techniques. Selection of the number and kinds of analytical techniques employed in each case will depend upon the magnitude of the monitoring program (e.g., number of stations and sampling frequency), the type of data collected, and the distributional characteristics of the data. As an example, a biomonitoring program of moderate complexity may include the following analyses of the benthic macrofauna:

- Calculation of community parameters such as diversity and species richness
- Graphical or tabular display of abundances of dominant organisms or indicator species
- Parametric or nonparametric statistical analysis of total organism density, densities of individual groups, species number, and community parameters
- Numerical classification of species abundance data (e.g., dendrogram).

Described below are analytical procedures recommended for use by the applicant in conducting the required comparisons. Emphasis has been placed on demonstrating the applicability of each analytical approach to the type of monitoring required. These descriptions are intended as concise introductions to techniques, each of which is the subject of numerous texts and technical papers. Many of the more important references are included under each description. The reference lists are not exhaustive, but they do provide a starting point for gaining access to the literature.

The formulation of plans to analyze results properly occurs during the development phase of the sampling design. Some statement must be made concerning the expectation of the type of data that will be developed and how that information will be used to address the issue of discharge impacts. If preliminary studies have identified substantial site-specific information, sampling objectives should be defined in detail. As discussed above in the section concerning sample frequency and replication, the ability of selected analytical techniques to detect differences in target parameters among monitoring stations must also be assessed.

Limitations are inherent in each of the analytical methods described below. Therefore, the inappropriateness of the singular use of any technique is stressed. Individual methods or statistical models may be of considerable utility in summarizing biological parameters; however, because important assumptions are specific to each method, a single analytical technique cannot accurately guide the interpretation of the monitoring program results.

Simple Graphic Displays--

Although statistical analyses of monitoring data are generally necessary for demonstrating compliance with the 301(h) biological criteria, graphical presentation of results provides an additional, and generally very useful, means of making comparisons among sampling sites. In many cases, the response of a biological parameter may be so pronounced that an effect is clearly evident in a graphical presentation and a detailed statistical analysis would not be necessary. Graphical displays are also important in presenting summaries of large amounts of data in a concise format.

The following types of graphical presentations are recommended for display of biomonitoring program results:

1. Community parameters (e.g., abundance, species richness, diversity) at sampling stations

2. Trellis diagrams or dendrograms of station similarities

3. Maps of faunal assemblages near discharge (generally in cases with large numbers of sampling sites in a heterogeneous environment).

Examples of appropriate graphical displays of biological data collected near marine sewage discharges are included in Figure 2. A discussion of techniques is included in Green (1979).

Parametric Techniques--

Parametric statistical techniques such as Student's t-tests and analysis of variance (ANOVA) are recommended for comparing measures of abundance and community structure among sampling stations. When it is hypothesized that outfall effects are evidenced by measurable differences between monitoring stations, these statistical models can be used to distinguish outfall-related impacts from natural variability in community structure.

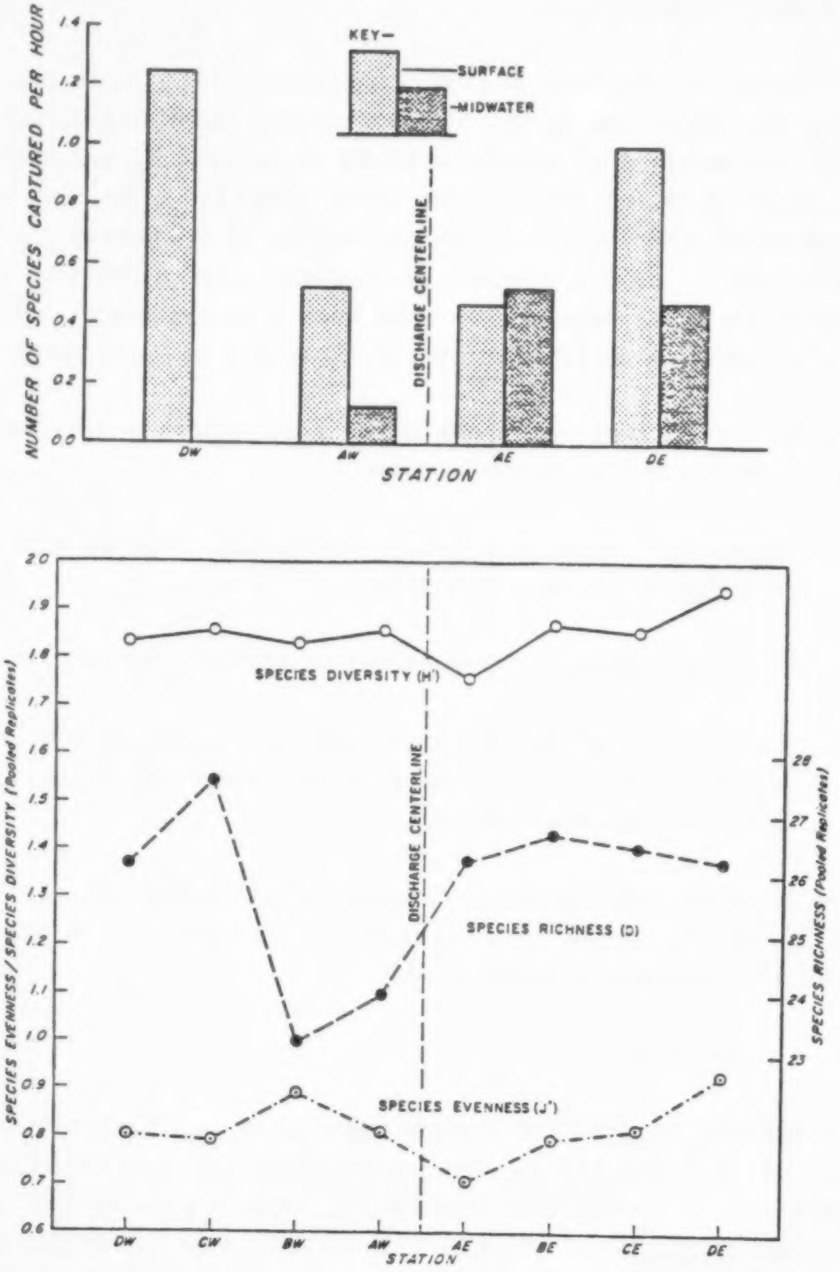


Figure 2. Examples of graphical displays of biological data from a marine sewage discharge site.

As the name implies, these tests involve null hypotheses concerning a statistical parameter of the variable being measured (e.g., population means). However, as such, they have specified assumptions concerning the distributional characteristics of the sample data. For ANOVA, it is assumed that the error terms of the variates in each sample are independent and normally distributed and that the sample variances are not different. Independence of error terms is primarily associated with adequacy of experimental design. The remaining two assumptions can be tested following data collection. If the data do not meet the assumptions, transformations can sometimes be applied to correct deviations from the assumed distributions. Discussions of transformations prior to ANOVA are found in Sokal and Rohlf (1969), Downing (1979), and Green (1979).

In many cases, a single transformation can correct both non-normal and heteroscedastic data. It is important to note, however, that deviations from normality, especially in cases of large sample size, will generally not influence the overall test results to the same degree as heteroscedasticity. Correlation of variances with means is a frequently encountered problem in samples of organism abundances. In many cases, such violations of the variance assumption can be corrected by a logarithmic transformation.

ANOVA is used to test the hypothesis that there are no differences in the biological observations made at different sampling stations. In addition to evaluation of a single factor (e.g., stations), ANOVA models are especially appropriate for evaluation of the importance of multiple factor level effects (e.g., depth, times) on the mean value of the dependent variable.

The t-test is statistically equivalent to ANOVA when only two samples are being compared. The t-test is appropriate for such two-sample comparisons; however, it should be emphasized that the test cannot be used to evaluate multi-sample hypotheses by testing all possible sample pairs. In such cases the probability of committing a Type I error is considerably higher than the designated level for each t test.

Discussions of the applications of ANOVA to biological data are found in Zar (1974), Sokal and Rohlf (1969), and Winer (1971).

If a significant effect is indicated in an ANOVA, an a posteriori multiple comparisons test should be used to identify where differences are located among the group means. The most commonly used a posteriori procedures are the Student-Newman-Keuls test (SNK), the least significant difference test (LSD), and Scheffe's test. Dunnett's test should be used if only the control mean is to be compared with all other group means rather than all possible comparisons. The characteristics of multiple comparison tests are described in Zar (1974).

Nonparametric Techniques--

If sample data do not meet the assumptions of parametric statistical tests, analogous nonparametric tests may be employed in the analysis of differences among stations. Nonparametric tests do not utilize null hypotheses associated with statistical parameters and there are typically no assumptions concerning the distribution of the variates. An additional advantage of nonparametric tests is that they can be used to test ordinal or nominal data in addition to numerical values.

Nonparametric tests have a lower power (i.e., $1-\beta$) than the analogous parametric procedures. For example, a nonparametric ANOVA has a power-efficiency of 95.5 percent when compared with the F test. Thus, nonparametric tests should not be applied if the sample data meet the assumptions of parametric techniques. Nonparametric tests are also unable to test interactive effects in the ANOVA model.

Examples of some common nonparametric tests and their applications are shown in Table 8. A comprehensive discussion of nonparametric techniques is provided in Siegel (1956) and Hollander and Wolfe (1973).

As for parametric ANOVA, an a posteriori test should be used following determination of a significant overall effect in the nonparametric analog of ANOVA. A multiple comparisons test for equal sample sizes analogous to the SNK test described in Zar (1974) and a procedure for unequal sample size is presented in Dunn (1964).

TABLE 8. EXAMPLES OF SOME NONPARAMETRIC STATISTICAL TESTS

Test	
Mann-Whitney U-test	Test of whether two independent samples are from same population (analogous to t test)
Kruskal-Wallis one-way ANOVA	Test of whether K independent samples are from different populations (analogous to F test)
Friedman two-way ANOVA	Test null hypothesis that K matched samples are from same population
X^2 test (or G-test)	Test of independence of frequencies in K samples.

Multivariate Techniques--

Multivariate numerical methods are used to reduce and order large matrices of data. They effectively summarize trends or patterns in the data that are not otherwise observed from visual examination or univariate analyses, and have been used to explore the interrelationships between sets of biological and concomitant physical-chemical observations. Their most common ecological application involves a search for patterns in measured biological variables which can be related to patterns in measured physical-chemical parameters. The goal in these analyses is to explain the effect of environmental variables on both community composition and structure. Examples of multivariate methods are discriminant analysis, multivariate ANOVA, ordination techniques, and numerical classification analysis.

Most multivariate tests have distributional assumptions analogous to the univariate case, the most important of which is equality of dispersion matrices. It is assumed that the variance-covariance matrices are independent of group means and are not different among groups. However, with increasing numbers of variables (p) in the multivariate case, the chance of detecting a significant difference becomes relatively high since there are 0.5 p (p + 1) variances and covariances. Heterogenous variance-covariance matrices will result in an increase in the probability of a Type I error, i.e., that a significant difference between groups will be indicated when one does not actually exist. In general, the potential for variance heterogeneity can be considerably reduced by use of equal replication, large sample sizes, and relatively few variables.

The increased probability of a Type I error is especially important when the overall power of multivariate tests is considered. As indicated by Green (1980): "When formal multivariate tests *are* made, their power (especially with many variables) is so great that a significant result is probable." Thus, the investigator should consider carefully the intended purpose(s) of multivariate analyses before they are applied. Specifically, it should be determined if the objectives are to reduce large data sets into a manageable format, to evaluate general relationships to environmental variables, or to test null hypotheses that no differences exist among sites.

Numerical classification methods are used to distinguish groups of entities (e.g., sample sites) according to similarity of attributes (e.g., species). Similarity of group attributes may be expressed using a variety of resemblance measures, including commonly-used similarity coefficients such as Jaccard, Bray-Curtis, Canberra metric, and Euclidean distance. Classification begins with the compilation of a matrix of similarity coefficients (index scores) between all possible pairs of entities. One of a variety of available clustering methods is then used to form graphical associations among entities to display groups of entities with similar attributes.

In most ecological applications of classification methods, sample collection sites are designated as the entities, and the relationship among sites is defined in terms of similarity of species occurrence. This approach is referred to as a normal classification, as opposed to an inverse classification in which species are selected as entities and their presence or abundance at the sample sites serves as the attribute. Analysis of the monitoring station data set using both normal and inverse classification methods, and the subsequent examination of normal-inverse coincidences using a two-way table are recommended.

A description of classification methodologies, the use of numerical classification, and an introduction to the literature concerning this analytical approach are presented in the EPA report, Application of Numerical Classification in Ecological Investigations of Water Pollution (Boesch 1977). Reviews of important classification strategies are given by Clifford and Stephenson (1975), Williams (1971), Sneath and Sokol (1973), and Goodall (1973); and examples of ecological applications can be found in Hughes and Thomas (1971), Boesch (1973), and Crossman et al. (1974).

Discriminant analysis summarizes multivariate information by weighting individual variables so as to maximize differences in groups of entities. This method describes differences between relatively homogeneous species-assemblages (defined, for example, in a numerical classification analysis) and facilitates identification of environmental variables which best separate these groups. An introduction to discriminant analysis is provided by Cooley and Lohnes (1971). Pertinent examples of the use of this method in the analysis of ecological data include Walker et al. (1979) and Green and Vascotto (1978).

Ordination refers to several multivariate techniques which are used to reduce the dimensionality of a data structure and to relate biological characteristics to environmental factors. The dimensionality is reduced by one of several methods which are designed to minimize the loss of information resulting from the reduction. In its most basic form, ordination may be used to group similar sites based on biological characteristics and to provide a graphical representation of between-group relationships. Ordination in this manner is analogous to the production of a dendrogram using numerical classification. A discussion of the use of reciprocal averaging ordination as a classification technique is presented in Culp and Davies (1980).

Principal component analysis (PCA) and factor analysis are techniques whereby axes scores in a reduced dimensional space are examined for relationships with abiotic variables. Variables displaying high correlations with component scores are assumed to be responsible for group separation based on biological characteristics.

The relative merits of alternative ordination methods are compared by Gauch and Whittaker (1972), and examples of environmental applications are presented in Smith and Greene (1976), Sprules (1977), Culp and Davies (1980), and Hughes and Thomas (1971).

Multivariate ANOVA (MANOVA) is analogous to univariate ANOVA, but includes measurement of more than one biological variable for each of several samples and all measured variables are tested simultaneously. The corresponding multivariate analysis of two samples is Hotelling's T^2 test. The basic assumptions are essentially the same as for the univariate case (i.e., normality and independence of error terms and homogeneity of within-group variance-covariance matrices). Although multivariate tests for variance heterogeneity are available, their application is not recommended by Green (1979) since they are more sensitive to the variance assumption than are the MANOVA tests. Transformations such as the logarithmic may be used to correct variance heterogeneity in the multivariate case so that relatively minor violations of the assumptions do not seriously affect the test results (Marriott 1974).

Biological Indices--

Indices have commonly been employed in impact assessment of biological communities because large amounts of multivariate data (i.e., abundances of individual species) can be reduced to a single number. Indices can be useful in this respect, but definite problems and limitations are associated with their use.

A primary problem is the failure of investigators to recognize the underlying assumptions and mathematical relationships of an index. By overlooking such considerations, an index may be selected, applied, and interpreted without a basic understanding of the properties of the biological community which are actually being measured. Comprehensive reviews of the assumptions and uses of diversity indices are provided in Green (1979), Pielou (1977), Sanders (1968), and Peet(1974).

An additional problem associated with indices is that they may be used extensively at the exclusion of other analytical or comparative methods which retain more of the available information. Indices may supplement multivariate techniques or analyses of individual taxonomic groups (see preceding sections), but field and laboratory studies have demonstrated that indices can be insensitive to rather intense biological change (Godfrey 1978; Swartz et al., 1980; Smith et al., 1979). Moreover, factors such as sample size, collection method, and time of year may have a profound influence on the value of an index (e.g. Hughes 1978). Therefore, standardization of sampling procedures is a prerequisite to conducting comparisons among index values.

Most indices commonly used in applied ecological studies are descriptions of community structure (e.g., species diversity, evenness, and richness). Other indices (e.g., Infaunal Trophic Index) incorporate additional descriptive characteristics for each species and provide for a description of community function. Several commonly-used diversity indices are listed in Table 9. Species richness (S) and Margalef's index (d) emphasize the number of species, are relatively simple, may provide valuable biological information concerning impact assessment, and are much less ambiguous than the information theory indices of Brillouin (H) and Shannon-Wiener (H'). H is the diversity value for a sample, while H' is an

TABLE 9. A LIST OF COMMONLY-USED INDICES OF DIVERSITY

Index	Symbol	Equation
Species Richness	S	number of species
Margalef	d	$S-1/\ln N$
Shannon-Wiener	H'	$-\Sigma \frac{n_i}{N} \log \frac{n_i}{N}$
Brillouin	H	$\frac{1}{N} \ln \frac{N!}{N_1!N_2!\ldots N_s!}$
Simpson's Index	SI	$1 - \Sigma \frac{n_i\ (n_i-1)}{N\ (N-1)}$

where:

S = number of species.

N = number of individuals.

n_i = number of individuals in the i^{th} species.

estimate for a random sample from a larger population. H' is used more frequently due to the complexity of calculating large factorials in H. However, for large numbers of individuals (N), the approximation, N (ln N-1), may be used for ln N!, and computer programs are available for exact calculation of H (Stauffer and Reish 1980). Pielou (1966) has suggested that H is generally more appropriate than H' since a truly random sample is required for estimation of H'. Simpson's Index is a measure of dominance which is determined primarily by a few of the most abundant species.

Another index available for impact assessment is the Infaunal Trophic Index (ITI) developed by Word (1978). The ITI is calculated as:

$$ITI = 100 - \left[33.33 \left(\frac{0n_i + 1n_2 + 2n_3 + 3n_4}{n_i + n_2 + n_3 + n_4} \right) \right]$$

where:

n_i is the number of individuals in trophic group i.

The ITI is based on the relative proportions of individuals in four trophic groups classified according to feeding types: suspended detritus feeders (I), surface detritus feeders (II), surface deposit feeders (III), and sub-surface deposit feeders (IV). ITI values correlate well with degree of organic enrichment, in that decreasing ITI values indicate increasing abundances of deposit-feeding organisms. The ITI is currently applicable to benthic macroinvertebrate communities at depths of 20 to 800 m (66 to 2,625 ft) in the Southern California Bight. Research is currently being conducted to determine applicability of the index to the Puget Sound area.

Since different ecological qualities are measured by the different indices, it is recommended that species abundance data be used to calculate at least three indices for each study: species number (S), combined number of species and evenness (H' or H), and dominance (SI). ITI would be an important additional parameter for studies in the Southern California Bight.

Indicator Species--

Many species display characteristic distributional responses to pollutant sources. Analysis of the occurrence of such species, referred to as "indicator species," may form a valuable component of the analysis of sewage discharge impacts. The primary value of the indicator species concept is that it allows for a considerable reduction in analytical complexity, since individual abundances of a few species are used to evaluate response of the community as a whole.

Indicator species may be divided into two categories:

- Sensitive organisms that display severely reduced abundances near pollutant sources

- Stress-tolerant or opportunistic species that display greatly enhanced abundances near pollutant sources.

In cases of organic enrichment, the first category of indicator species is generally composed of suspension-feeding organisms such as *Ampelisca* spp. (Amphipoda) and *Amphiodia* spp. (Ophiuroidea). Reduced abundances of these species may also indicate high sensitivity to toxic chemicals contained in the effluent.

The second category of indicator species, those having high abundances in polluted areas, has received more intensive study than the former group. Such species may have a high tolerance to organic enrichment or toxic chemicals in addition to an opportunistic life strategy (e.g., short generation time and/or lack of larval dispersal). These attributes enable them to exploit available resources in the absence of nontolerant competitors or predators following habitat disruption or pollutant stress. A list of some polychaetous annelids that have been associated with pollutant sources is provided in Table 10.

Although most of the species in Table 10 have been observed in high abundances in very polluted areas, indicator species may also be used to detect areas of moderate pollutant stress or transitional regions between polluted and normal areas. Word et al. (1977) characterized species

TABLE 10. LIST OF SOME COMMON POLYCHAETES
THAT HAVE BEEN ASSOCIATED WITH MARINE
OR ESTUARINE POLLUTION

Capitella capitata

Polydora ligni

Streblospio benedicti

Scolelepis fuliginosa

Schistomeringos rudolphi

Dorvillea articulata

Heteromastus filiformis

Mediomastus ambiseta

M. californiensis

Eteone longa

Ophiodromus spp.

Cirriformia tentaculata

Neanthes succinea

N. caudata

Source: Pearson and Rosenberg (1978).

indicative of polluted and transitional areas of the Southern California Bight. The clam *Parvilucina tenuisculpta*, the annelids *Tharyx* spp. and *Mediomastus californiensis*, and the ostracod *Euphilomedes* spp. are present in low abundances in control areas and reach much higher abundances (based on absolute abundances and proportion of total infauna) in areas of organic enrichment.

The primary limitation of the indicator species concept is that it should be used only with a full consideration of the normal distributional patterns and environmental associations of the species. This is especially true in estuarine environments where salinity fluctuations and high organic inputs may result in natural elevated abundances of opportunistic species. In the marine environment, areas of natural organic accumulation (e.g., submarine canyons, kelp beds) may also have high abundances of opportunistic organisms.

Before using an indicator species as part of a 301(h) monitoring program, several types of information should be developed:

- Natural abundances of species in control areas
- Response of species to environmental conditions other than pollutant stress
- Observed response of species to pollutant sources in the biogeographic zone.

The primary requirement is that species abundance be adequately described for control conditions. Proper selection of control sites will ensure that any observed differences in the abundances of indicator species are due to the discharge in question and not to natural or other anthropogenic stresses.

Data Reporting

The information presented as part of a biological monitoring program should consist of three general kinds:

- Methods
- Study results and summary of findings
- Data reports.

A discussion of study methods should be presented in each report, including such aspects as station locations, sampling procedures, sampling processing, subsampling, quality control, and analytical methods. Procedural details should be provided unless a standardized technique is used, in which case a reference should be included. Citations should also be provided for all taxonomic references used for organism identifications.

The presentation of study results should include a general characterization of the biological communities sampled. Emphasis should be placed upon descriptions of both spatial and temporal trends in community structure and function. Specific comparisons should be conducted for all biological criteria contained in the 301(h) regulations (e.g., ZID boundary vs. reference communities). Where statistical analyses are performed, the report should include details of the results. For example, in case of ANOVA, the entire ANOVA table should be presented, not just a statement concerning the significance level of the F value. Biological variables (e.g., species abundances, diversity, richness) should be presented in graphical or tabular format as means and their 95 percent confidence intervals ($X \pm t_{(n-1)} S_X$) for each sampling station.

Each monitoring report should include copies of the field collection logs and laboratory sample counting forms. The data provided should include the actual numbers of each species counted in each sample and the calculated areal or volumetric abundance of each taxon. Sufficient detail should be provided to allow for verification of analyses conducted as part of the monitoring program, or for reanalysis of the submitted data.

CHAPTER V

QUALITY CONTROL

The U.S. EPA policy on quality assurance and control (EPA Administrator memoranda dated May 30, 1979, and October 14, 1981) stipulates that every monitoring and measurement project must have a written and approved quality assurance project plan. This requirement applies to all environmental monitoring and measurement efforts mandated or supported by EPA. A quality assurance project plan will specify the policies, organization, objectives, and functional activities designed to achieve data quality goals of individual projects or continuing operations. The monitoring programs of 301(h) permittees are covered by this policy.

Several EPA publications are available from the Office of Monitoring Systems and Quality Assurance, ORD, USEPA, Washington, D.C., 20460, on the subject of quality assurance. EPA Publication QAMS-005/80, for example, describes 16 elements which should be included in all quality assurance project plans. That publication establishes criteria for plan preparation, including procedures to be used to document and report precision, accuracy, and completeness of environmental measurements. In addition, the following paragraphs provide guidance on quality control procedures specific to 301(h) monitoring programs. The guidance provided below focuses primarily on water quality monitoring and toxics control monitoring. Additional guidance on quality assurance and control procedures is provided in Chapter IV, Biological Monitoring, particularly the subsection on benthos.

APPROACH AND RATIONALE

In 301(h) monitoring programs, differences, or the lack of differences, among samples must be demonstrated. When the differences to be defined are small relative to background concentrations (as is the case for many parameters), it becomes imperative to know and control the uncertainty associated with sampling and analytical procedures. This is necessary to distinguish true field variability from that induced by sampling or laboratory procedures.

It is imperative to have qualified personnel who are conscientious and properly trained. Such personnel, using adequate equipment and procedures, will help to ensure a clear understanding of sampling and analytical variability relative to true field variability. A well defined quality assurance/quality control program should be designed as an integral part of the 301(h) monitoring program. The quality assurance/quality control program should have as its basis a simple, but rigorous, quantitative approach which can be applied consistently for the control of error. The sampling and analytical procedures selected should provide feedback so that those performing the analyses can promptly detect and correct procedural problems. Thereby, the uncertainty regarding field measurement variability will be minimized.

The section which follows describes quality control procedures associated with field activities. A quantitative error analysis procedure is then presented in detail with graphical techniques for detection of increases in analytical error over time. Finally, quality control procedures specific for toxic pollutant analyses are briefly described.

FIELD ACTIVITIES

It is important that field activities be well planned in advance and that as many decisions as possible be made before field sampling commences. Problems are encountered in coastal work which do not occur normally during the sampling of inland waters. Highly conductive salt mists frequently cause problems with electronic instrumentation. There is generally a lack of nearby fixed points (landmarks, permanent buoys) from which to locate sampling stations. Waves and swells make the sampling vessel unsteady, cause motion sickness, and make work difficult.

Field activity quality control can be subdivided into three categories: 1) accurate station location, 2) proper sampling procedures, and 3) proper documentation of sampling efforts. Station location can best be assured by use of redundant navigation systems. For example, the use of portable electronic range positioning systems plus the use of a sextant in the horizontal mode would form such a redundant system. The two separate procedures together help to minimize error. The installation onshore of line of sight targets can greatly aid the positioning effort. Sampling crews can line up the vessel from these first and then go to the position fixing techniques for improved resolution. Finally, following sampling at a station, the bottom should be sounded using a manual (non-electronic) system, such as a lead line. The known depth (from charts and prior measurements) and the lead line results can be compared for a rough check on position.

There are two general types of sampling: 1) in situ measurements, and 2) the collection of water, sediment, and biological samples for subsequent analysis. In situ measurements are normally made with electronic systems (in situ observations by diver-biologists are discussed in Chapter IV, Biological Monitoring). Calibration of these systems should be done before and after each series of field measurements. Probe systems, except perhaps for the hydrogen-ion electrode, are often unreliable in terms of absolute accuracy and are best used in a differential sense (e.g., to measure changes in parameters with depth while profiling at a given station).

Errors in sampling depth are caused by several factors including ship motion and drag on the underwater sampling equipment. In high currents the drag on instrumentation and cables may result in significant errors if sampling depth is determined solely by the length of cable underwater. Drag can be mathematically corrected for only if the current profile and the drag coefficients for the instrumentation are known. The displacement of the sampling device(s) from the desired position is calculated by either summing the moments about the point where the cable enters the water or by making a free body analysis of the forces on the sampling device and cable.

Many multi-probe in situ measurement systems incorporate depth measurement by use of pressure transducers. The accuracy and precision of such systems must be periodically checked. This is most easily done in calm slack water. Precision is determined by multiple measurements at the same depth. Accuracy is evaluated by comparison to measurements made with a heavily weighted lead line.

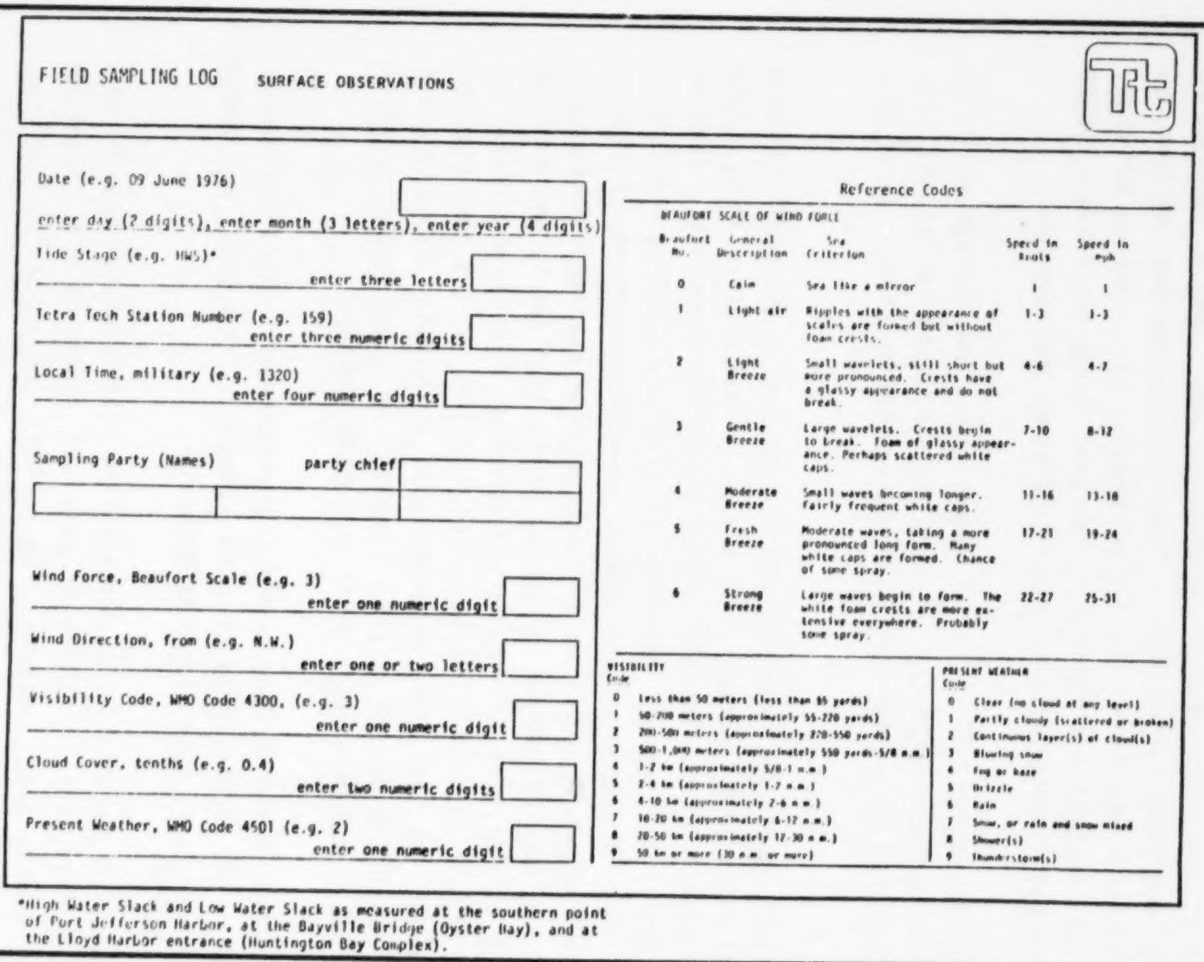
Dissolved oxygen probe systems should be calibrated using the modified Winkler titration technique. Profile measurements of salinity and temperature are used to determine water column stability and are an aid in the prediction of plume behavior. Salinity probe systems offer moderate accuracy but should be cross-checked by discrete water samples analyzed by induction type laboratory salinometers. Temperature probes may, at best, be accurate to within one-tenth to three-tenths of a degree Celsius. Temperature probe systems are rarely linear over large temperature ranges and must be checked against research grade laboratory thermometers.

Water quality sample collection, preservation, and storage should be performed in accordance with the procedures discussed in Chapter III. Procedures for taking biological samples are presented in Chapter IV of this document.

An important aspect of receiving water sampling is the order in which procedures are executed upon occupying a station. Vessel positioning should first be completed. (Vessel location should be checked frequently.) Surface observations should be made and recorded on standard sampling sheets (see for example Figure 3). Water column samples are then collected. Following that, the water column is profiled using in situ measurement techniques. Next, any benthic samples should be taken. Only then should the depth be sounded using a lead line or equivalent physical technique. Finally, documentation should be completed before proceeding to the next station. The major purpose of the above sequencing of activities is to prevent detritus, resuspended during bottom sampling or depth sounding, from contaminating water column samples or in situ measurements.

Documentation should include surface observations, sample log sheets, and data sheets with results from in situ sampling. Waterproof preprinted forms bound as log books have proven to be useful.

It should be reemphasized that the sampling program design must be well detailed and that as many decisions as possible should be made before the sampling crew starts their efforts. In coastal and estuarine sampling, unpredictable problems will occur and will demand the immediate attention of the sampling crew. Time for this type of on-site decision making must be available.



FIELD SAMPLING LOG SURFACE OBSERVATIONS

Date (e.g. 09 June 1976)

enter day (2 digits), enter month (3 letters), enter year (4 digits)

Tide Stage (e.g. HWS)*

enter three letters

Tetra Tech Station Number (e.g. 159)

enter three numeric digits

Local Time, military (e.g. 1320)

enter four numeric digits

Sampling Party (Names) party chief

Wind Force, Beaufort Scale (e.g. 3)

enter one numeric digit

Wind Direction, from (e.g. N.W.)

enter one or two letters

Visibility Code, WMO Code 4300, (e.g. 3)

enter one numeric digit

Cloud Cover, tenths (e.g. 0.4)

enter two numeric digits

Present Weather, WMO Code 4501 (e.g. 2)

enter one numeric digit

Reference Codes

BEAUFORT SCALE OF WIND FORCE

Beaufort No.	General Description	Sea Criterion	Speed in Knots	Speed in mph
0	Calm	Sea like a mirror	1	1
1	Light air	Ripples with the appearance of scales are formed but without foam crests.	1-3	1-3
2	Light Breeze	Small wavelets, still short but more pronounced. Crests have a glassy appearance and do not break.	4-6	4-7
3	Gentle Breeze	Large wavelets. Crests begin to break. Foam of glassy appearance. Perhaps scattered white caps.	7-10	8-12
4	Moderate Breeze	Small waves becoming longer. Fairly frequent white caps.	11-16	13-18
5	Fresh Breeze	Moderate waves, taking a more pronounced long form. Many white caps are formed. Chance of some spray.	17-21	19-24
6	Strong Breeze	Large waves begin to form. The white foam crests are more extensive everywhere. Probably some spray.	22-27	25-31

VISIBILITY

Code	
0	Less than 50 meters (less than 55 yards)
1	50-200 meters (approximately 55-220 yards)
2	200-500 meters (approximately 220-550 yards)
3	500-1,000 meters (approximately 550 yards-5/8 n.m.)
4	1-2 km (approximately 5/8-1 n.m.)
5	2-4 km (approximately 1-2 n.m.)
6	4-10 km (approximately 2-6 n.m.)
7	10-20 km (approximately 6-12 n.m.)
8	20-50 km (approximately 12-30 n.m.)
9	50 km or more (30 n.m. or more)

PRESENT WEATHER

Code	
0	Clear (no cloud at any level)
1	Partly cloudy (scattered or broken)
2	Continuous layer(s) of cloud(s)
3	Blowing snow
4	Fog or haze
5	Drizzle
6	Rain
7	Snow, or rain and snow mixed
8	Shower(s)
9	Thunderstorm(s)

*High Water Slack and Low Water Slack as measured at the southern point of Port Jefferson Harbor, at the Bayville Bridge (Oyster Bay), and at the Lloyd Harbor entrance (Huntington Bay Complex).

Figure 3. Oceanographic surface observations log sheet.

QUANTITATIVE ERROR ANALYSIS

This section presents techniques for error analysis. The focus is on those parameters for which discrete water samples are taken in the field and analytical work is performed in the laboratory. The error analysis procedures are also applicable to in situ measurement. Analytical precision is then determined by multiple measurements made over a short time period on a discrete water sample, a procedure analogous to the splitting of samples described in the following section. Accuracy can be determined by field (e.g., probe) and laboratory analysis of the same sample, and the results analyzed in the fashion described below for spiked samples (with the concentration added set to zero).

Quantitative error analysis is based on the premise that every measurement is subject to uncertainty. Uncertainty in water quality measurements arises from the systematic bias and limited precision of sampling and analytical procedures and from heterogeneity in the water column at sampling sites. The quantitative determination of this uncertainty serves as the basis of two essential steps in the control and assurance of measurement quality:

- The routine monitoring of analytical precision and accuracy
- The presentation of results in a way that informs the reviewers of the uncertainty of measurements and the confidence which may be placed in conclusions drawn from the results.

The first step in the quantitative analysis of errors is the verification of a discharger's ability to produce analytical results which are sufficiently accurate and precise to meet the specifications stated in the table of recommended analytical methods in Chapter III. This determination is made prior to the initiation of routine monitoring for each water quality parameter to be measured. The recommended procedures for determining precision and systematic bias are summarized in Table 11.

TABLE 11. RECOMMENDED PROCEDURES FOR DETERMINATION OF SYSTEMATIC BIAS AND PRECISION IN ANALYTICAL METHODS

Precision	Systematic Bias
• The precision is determined at four concentrations: one concentration near the limit of detection of the procedure, two intermediate concentrations, and one concentration near the upper limit of application of the method.	• Add known amounts of the analyte to the low and one of the intermediate concentration samples used to determine precision. Enough additional material is added to double the lower concentration and to raise the intermediate concentration to 75 percent of the analytical limit.
• Seven subsamples are analyzed at each of the four concentrations.	• Seven subsamples of each spiked sample are analyzed.
• For procedures using analytical instruments: 1) conduct analyses over a two-hour period. 2) run samples in the sequence high, low, intermediate, intermediate. Repeat sequence seven times.	• Systematic bias is reported as percent recovery at the final concentration of the spiked sample using the mean of the seven analyses.
• Report the mean, standard deviations (S_a) and number of samples analyzed at each concentration.[a]	

It is strongly recommended that actual samples be used in these analyses in order to include the effects of naturally occurring interferences.

These procedures can be adapted to nearly all the analytical methods specified in the monitoring program, except those using gas chromatography - mass spectometry.

These procedures for determination of precision and systematic bias are taken from U.S. EPA "Handbook for Analytical Quality Control in Water and Wastewater Laboratories" (1972).

The initial verification of analytical competence is followed by the continuous monitoring of analytical quality by each discharger or their associated laboratory. It is essential that an unsatisfactory analytical procedure be quickly identified and corrected in order to prevent the accumulation of inaccurate data. This is accomplished primarily through the routine analysis of split and spiked samples and the use of quality control charts (as shown in Figure 4).

Laboratory analytical precision should be checked by splitting a percentage of homogeneous field samples into replicates and analyzing all subsamples. Sample splits should be made in the field. Laboratory personnel should be kept unaware of which samples have been split. Ideally, splits should not be analyzed in succession. For each split sample, the individual measurements and the range are reported for each parameter. If possible, a double blind procedure should be used.

The accuracy, which includes precision and systematic bias, of an analytical procedure is routinely monitored by spiking a field sample with a known amount of the analyte (a standard addition). As with the split samples, personnel performing the analyses ideally should remain unaware of which samples have been spiked. The deviation from stoichiometric behavior of spiked samples is calculated from the following expression:

$$E = X_s - (X_o + A) \qquad (1)$$

where:

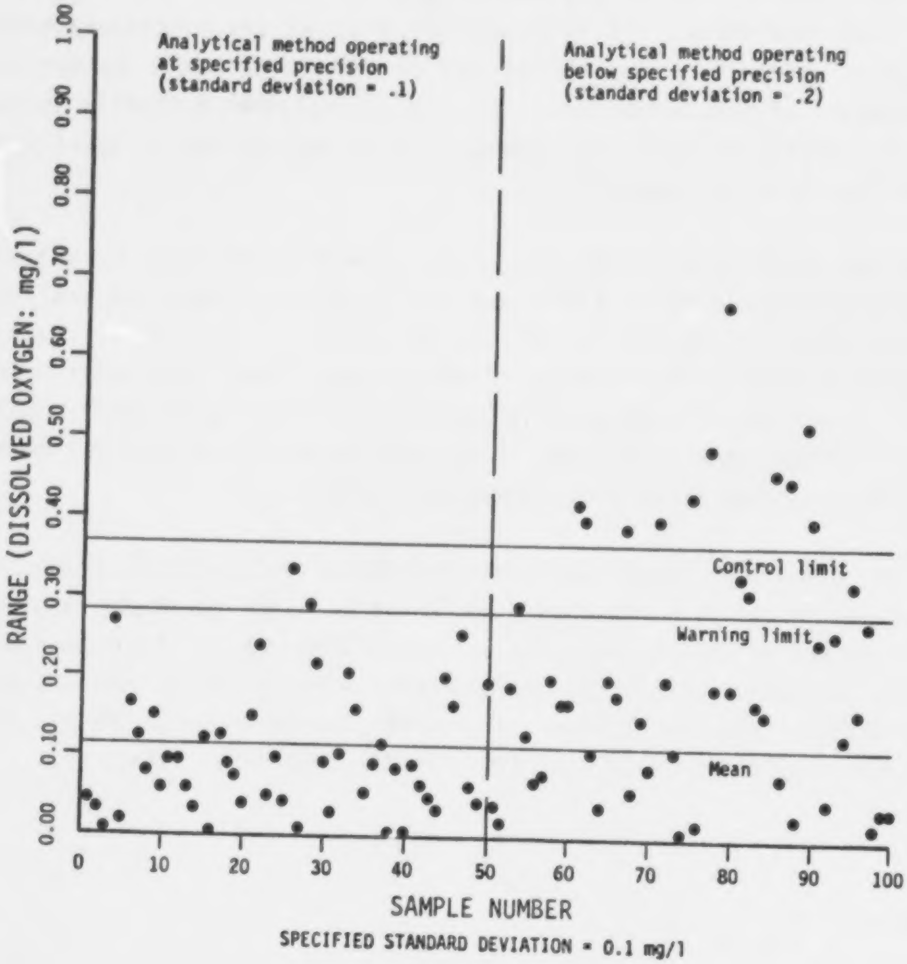
E = deviation from stoichiometric behavior

X_s = measured concentration of spiked samples

X_o = measured concentration prior to addition of spike

A = increase in concentration due to spike.

The deviation from stoichiometric behavior of each spiked sample should be reported along with the spiked and unspiked sample concentrations.



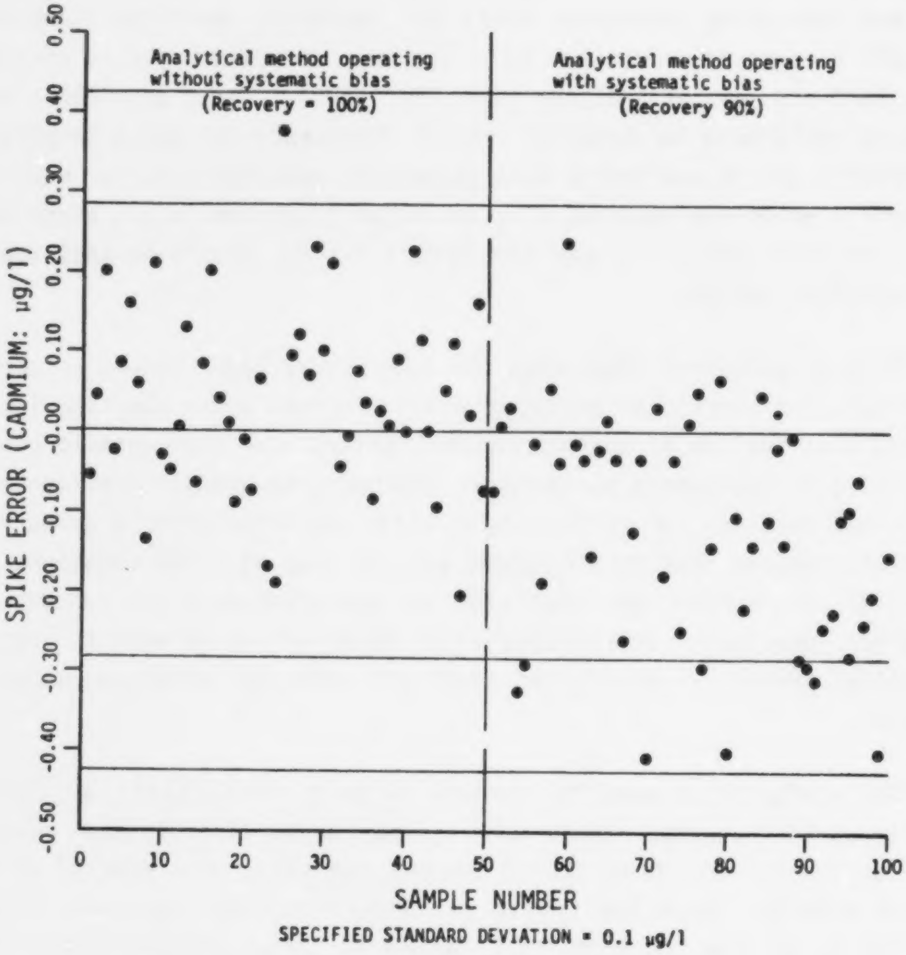
NOTE:
This figure illustrates how a decrease in measurement precision would affect the distribution of data plotted on a precision control chart. In actual practice, as soon as the loss of precision is detected--by about sample 62 in this case--the cause of the decreased performance can be isolated and corrected. (Precision control data were generated using a mean oxygen concentration of 5.0 mg/l.)

Figure 4. Example Precision Control Chart.

The frequency with which split and spiked samples are analyzed must represent a balance between reducing the likelihood of generating inaccurate data and increasing laboratory costs for analyzing additional samples. The U.S. EPA recommends that one split and one spiked sample be analyzed for every 10 field samples analyzed (U.S. EPA 1979b). Those applicants who wish to do so would have to justify a lower frequency of split samples. This requirement can be applied to most parameters measured daily or weekly. For parameters which are measured only at longer intervals (e.g., every month or more), at least one split and one spiked sample should be analyzed during each sampling period.

Project personnel other than the laboratory staff should be careful to distribute the additional analyses over time and among sampling locations. An even distribution of quality control effort over time permits continuous monitoring of laboratory performance and ensures greater confidence in the analytical results. Distributing quality control efforts among sampling locations ensures that interferences present only at a few locations will be detected and enables the precision in measurement to be determined for different locations. It is especially important to be able to distinguish analytical precision associated with effluent and receiving water sample analyses.

The analysis of quality control data is facilitated by the use of quality control charts. Individual measurements of analytical measurement range and deviations from stoichiometric behavior are plotted on a graph marked with the expected limits of deviation from the mean. Standard practice is to draw "control limit" lines at three standard deviations from the mean and "warning limit" lines at two standard deviations from the mean. Since these correspond to 99.7 and 95.5 percent confidence limits, the analytical procedure is considered out of control or potentially out of control when the limits are exceeded more frequently than one in 370 and one in 20 samples, respectively. In some cases, a developing analytical problem appears as a diverging trend on a control chart even before the control limits are exceeded. Examples of control charts illustrating cases when the analytical procedure is under control and when it is not are given in Figures 4 and 5.



NOTE:
This figure illustrates how the presence of a systematic bias would affect the distribution of data plotted on an accuracy control chart. In actual practice, as soon as the systematic bias was detected--before sample 60 in this case--the cause of the decreased performance would be isolated and corrected. (Accuracy control data were generated using a mean cadmium concentration of 0.8 µg/l [analytical standard deviation = .1 µg/l] with 1.0 µg/l added as spikes).

Figure 5. Example Accuracy Control Chart.

The construction of quality control charts is a simple process. The expressions necessary to calculate the limit lines are summarized in Table 12. Note that in many cases where the analytical variance shows a concentration dependence, the quality control parameters can be adjusted to account for this dependence. If most measurements of a parameter remain within a small range (e.g., ± 15 percent of the mean value), the assumption of constant variance is sufficiently accurate for control chart purposes even when the variance is concentration dependent.

For the purposes of verifying compliance with the precision and accuracy specifications, quality control data should be plotted on control charts derived from these same specifications. If a laboratory's precision or accuracy is substantially better than required, additional limit lines corresponding to the observed performance could be added to assist in monitoring analytical performance more closely. Methods for generating control charts from laboratory data are described in U.S. EPA (1979b) and American Society for Testing and Materials (ASTM) (1951).

Analysis of unknown standards for all parameters should be performed at least once a year in order to identify systematic error not detectable by spiked samples. It is recommended that the dischargers or their selected laboratories participate in the U.S. EPA interlaboratory testing program. The interlaboratory correlation technique of Youden (1960) could be used for comparison of results.

As a final check, all submitted data should be critically reviewed. It may be requested that unusually high or low measurements be reanalyzed.

The second function of quantitative error analysis is to facilitate the presentation of data in a way that permits open inspection of their certainty or uncertainty. This requires quantitative estimates of both the variability introduced in measurements by analytical imprecision and field heterogeneity and the limits of sensitivity of analytical methods.

The variability inherent in the analytical procedure is determined from the results of split sample analysis. If the variance is relatively constant over the range of measured concentrations, a pooled estimate of the analytical variance can be made using the following expression:

TABLE 12. SUMMARY OF EXPRESSIONS NECESSARY TO CONSTRUCT CONTROL CHARTS

	Quality Control Data Used	Dependence of Analytical Variance on Concentration	Quality Control Parameter to Be Plotted on Y - Axis	Control Chart Lines[a]
PRECISION	Ranges determined for split samples, R	S^2_a independent of concentration $\overline{S^2 = S^2_a}$	R	**Central Line** R = 0.28 x S **Warning Limit Line** R = 2.834 x S **Control Limit Line** R = 3.686 x S
		S^2_a dependent on concentration[c] $\overline{S^2 = S^2_a/X^n}$	$\frac{R}{\bar{X}^{n/2}}$	
ACCURACY[b]	Deviation from Stoichiometric behavior determined for spiked samples, E	S^2 independent of concentration $\overline{S^2 = S^2_a}$	E	**Central Line** E = 0 **Warning Limit Line** E = ± 2.828 x S **Control Limit Line** E = ± 4.243 x S
		S^2_a dependent on concentration[c] $\overline{S^2 = S^2_a/X^n}$	$\frac{E}{\sqrt{(X^n_s + X^n_o)/2}}$	

Definition of Variables:

S = concentration independent measure of precision.

S_a = specified or observed standard deviation of analytical procedure at analyte concentration, x.

X = concentration of analyte.

n = power dependence of variance on concentration, e.g., for constant coefficient if variation ($S_a/X = S$), N = 2.

[a] Control chart lines derived from equations in ASTM (1951).

[b] Accuracy control lines represent limits of deviation from stoichiometric behavior which is attributable to analytical imprecision.

[c] In some cases, the dependence of the variance on concentration can be adequately represented by the following expression:

$$S^2_a = (X^n)(S^2).$$

When this is the case, a plot of log (S_a) vs. log (X) will form a straight line from which both S and n may be determined. The equation of the line is:

$$\log (S_a) = \frac{n}{2} \log (X) + \log (S).$$

$$s^2_a = \frac{1}{2N_s} \bullet \sum_{i=1}^{N_s} R^2_i \qquad (2)$$

where:

s^2_a = analytical variance

N_s = number of split samples pooled

R_i = range of subsample measurements for sample i split n ways.

If the variance is concentration dependent, a different parameter, e.g., the coefficient of variation, can be averaged.

The variability in measurement caused by field heterogeneity is quantitatively determined by the analysis of replicate field samples. Two sampling strategies should be considered:

1. Collect replicate field samples and analyze multiple subsamples of each. Analysis of Variance (ANOVA) is then applied to determine the contribution of field heterogeneity to the variance. The drawback of this approach is that it requires a large number of analyses for even minimum resolution power, e.g., 4 x 4 = 16.

2. Collect replicate field samples and analyze each only once. The field-induced variance is estimated using the principle of variance additivity. This approach makes additional assumptions concerning the analytical variance but requires many fewer analyses to be made, e.g., 4 x 1 = 4.

Since the application of the first approach is well understood [see for example Sokal and Rohlf (1969)], only the second will be described in detail here.

The second approach is based on the assumption that an independent estimate of the analytical variance exists which is applicable to the conditions under which the field replicates are analyzed. If this is the case, the field variability can be estimated from the following expression [American Chemical Society (1980)]:

$$S^2_f = S^2_r - S^2_a \qquad (3)$$

where:

S^2_f = variance due to field heterogeneity

S^2_r = variance of replicate field samples

S^2_a = analytical variance.

Equation 3 can be applied using the pooled estimate of analytical variance (Equation 2), if one the following conditions is met:

1. The analytical variance is not strongly dependent on analyte concentration or background interferences.
2. The concentration of analyte in samples used to compute the pooled or initial analytical variance is similar to that in the field replicates.

Replicate sampling should be conducted at all field stations where measurements are to be used in comparisons. Analysis of replicate sample data is necessary for assessing the reliability of such comparisons. When replicate sampling at all stations is not feasible, replication is required for at least one station from each group of stations which may reasonably be assumed to have similar amounts of field heterogeneity. As a minimum, replicates should be collected at one ZID boundary station and one reference (control) station.

The number of replicates to be collected at each station depends on the use intended for the data. For example, more replicates are required to make a meaningful comparison of variances than are required to compare mean values. Appropriate statistical methods should be applied to each case. Sevenfold replication is currently recommended by U.S. EPA (American Chemical Society 1980). Depending upon circumstances, overall project design, and the applicant's resources, fewer replicate determinations may be accepted.

If possible, replicate samples should be taken at all specified stations within the first year of the sampling program during a period of maximum natural variability. This would provide information on field heterogeneity to be utilized during the sampling program in-progress review (review of first annual report). At that time, the replication program should be evaluated and re-designed if necessary. In addition, the knowledge of field variability can be applied to design a composite sampling scheme for all stations. By analyzing one sample formed by combining and homogenizing a number of replicate samples, the uncertainty in the measurement due to field heterogeneity can be reduced without performing any additional analyses. The following expression defines the number of replicates required to obtain a confidence limit of E for a mean value:

$$N_r = (t \cdot S_f/E)^2 \qquad (4)$$

where:

t = appropriate value of Student's distribution

N_r = number of replicates required

S_f = field variability: $S_f >> S_a$.

When the condition of negligible analytical variance is not met, composite sampling is of little value.

In addition to the analytical and field variances, knowledge of the limits of quantitation and detection is essential to the assessment of measurements at trace levels. The method recommended by the American Chemical Society (1980) is based on the analytical variance determined using a field blank as follows:

$$L_D = 3 \cdot S_B$$

$$L_Q = 10 \cdot S_B$$

where:

L_D = limit of detection

L_Q = limit of quantitation

$S_B = (S_t^2 + S_b^2)^{1/2}$ where S_t and S_b are the standard deviations of an instrument response to replicate instrument runs of a single analyte - containing sample and a blank sample, respectively.

For a more detailed analysis of limits of detection and quantitation, see Currie (1968).

TOXIC POLLUTANT ANALYSIS

Assuring the quality of toxic pollutant analyses requires numerous precautions beyond those necessary for other water quality parameters. Most of the additional quality control procedures are necessitated by the greater complexity of analytical instruments used for toxic pollutant analyses and the risks of sample contamination. Requiring all toxics analyses to be conducted by U.S. EPA certified laboratories helps assure the adequacy of internal quality control practices. Each laboratory should be practicing the quality assurance steps outlined in the source documents for each procedure as well as appropriate methods in the quantitative error analysis portion of this chapter. Special quality control procedures for toxics not dealt with in the above sources are the concern of the remainder of this section.

Extra care in sample handling is required for toxic pollutant analysis since these pollutants generally occur in trace concentrations and frequently are unstable. Samples should be stored in the dark to avoid photochemical decomposition. Storage at reduced temperatures, as specified in Chapter III, minimizes the rate of other degradative chemical reactions.

Exposure of the sample to the atmosphere should be minimized in order to avoid loss of volatile compounds.

Sample bottles must be clean and made of materials which will not contaminate the samples. Plastic or glass bottles must be specified depending upon the analyses to be performed on the sample. Caps for all glass bottles used to store toxics samples should be teflon lined. Glass bottle and cap liners should be cleaned with chromate cleaning solution and successively rinsed with distilled water and several portions of the appropriate spectral grade redistilled organic solvent. Bottle caps should be washed with detergent and rinsed using the same steps described above. Plastic sample bottles should be cleaned with detergent or concentrated hydrochloric acid and rinsed with distilled water.

Since many organic substances are strongly sorbed by particulate matter, it is essential that effluent samples contain a fraction of suspended solids representative of the entire waste stream. This should be considered in the selection of sampling devices and locations. The variability in measurements introduced by sampling techniques, together with the variability caused by effluent heterogeneity, are considered below.

The assessment of the significance of toxic pollutant measurements depends on a knowledge of the variability in measurements introduced by sampling technique and site heterogenity. This variability is determined by the analysis of replicate samples. The rationale used to specify the number and frequency of replicate samples is contained in the Quantitative Error Analysis portion of this chapter. Replicate samples should be collected from the effluent stream and in most cases from the sediments of at least one field station. Replication should be carried out at least once a year during the period of highest toxic pollutant levels.

The qualitative and quantitative analytical capabilities of a laboratory should be verified as well. One effluent sample spiked with each of the routinely monitored substances (except dioxin) should be analyzed with each group of samples. A blank (glass doubly-distilled water) should be analyzed along with each group of samples screened for priority pollutants.

APPENDIX A

OCEANOGRAPHIC METHODS

This appendix is intended to provide background information and guidance on collection of oceanographic data. Types of current meters and their proper use are summarized. The use of drogues, drifters, and dye studies are also reviewed. In addition, specifications for field use of current meters, drogues, drifters, and dye studies are discussed. Positioning methods are briefly reviewed.

CURRENT METERS

Uses of Current Meters

Current meters are used to measure the variation in current speed and direction at a fixed location with time. Since each meter collects data only at a single point, several current meters may be required to establish the velocity field over depth and over a given area. A typical fixed current meter array may contain a meter near the bottom, one near the surface, and one at mid-depth. Short-term current measurements can be taken from a boat with a meter lowered over the side and connected by cable to an on-deck recorder. Longer term in-situ measurements may be obtained by installing meters with self-contained recording devices (e.g., magnetic tape, film, and strip charts) on a mooring system anchored to the bottom. Current speed and direction may be recorded either continuously, or at specified time intervals. Vector averaging meters electronically average the measured velocity components over specified sampling intervals, and provide the average current over each interval as output.

Different current meters are designed to work in different operating environments. Meters designed to operate at greater depths must be able to withstand higher pressures, and must be capable of accurately measuring relatively low current speeds. Meters designed to operate in shallow nearshore coastal environments must be able to accurately measure currents in the presence of wave action. The effects of waves include both oscillatory water particle orbits in the immediate vicinity of the meter, and the pumping action of the mooring lines where a surface or subsurface buoy is subject to wave-induced motions.

Types of Current Meters

The current meters presently in use may be classified as mechanical, electromagnetic, or acoustic. Mechanical current meters include Savonious rotors, ducted impellers, drag inclinometers, and propeller-type meters. Savonious rotor current meters utilize a unidirectional rotor on a vertical axis of rotation and a vane which senses the horizontal direction of flow. Although these meters have been used for many years in oceanographic work, they are not suited for operation in shallow water environments which are exposed to wave and swell activity. In such waters the oscillatory velocity components due to the orbital motions of waves are significant. Since the rotor turns in only one direction, the oscillatory velocity components due to waves are recorded as a rectified velocity input. The rectified record cannot be adequately resolved into the oscillatory components since the directional response of the meter is slow relative to the wave motions, and its response to deceleration is different than its response to acceleration (Horrer 1968). As a result, the velocity record is distorted and never reaches zero velocity, even in the presence of wave action alone. This makes it difficult to distinguish the steady component of the current from the oscillatory component due to waves. Savonious rotors should, therefore, only be used in deep waters below the influence of wave action or in areas where the current speeds are high and the waves very small.

Two types of ducted impeller current meters are available which attempt to eliminate the problems associated with current measurements in the presence of waves. One type manufactured by Endeco is a neutrally buoyant meter with a bidirectional impeller which is attached to the mooring line by a tether several feet in length. The tether and neutral buoyancy allow the meter to move with wave-induced water particle orbits, so these short-term velocity oscillations are not recorded. The meter is said to orient itself in a wave field so that only the mean current is measured. A tilt compensation mechanism keeps the meter horizontal (Brainard and Lukens

1975). Another horizontal ducted impeller meter manufactured by Bendix has a long boom vane to keep the meter oriented in the direction of mean flow, preventing it from rotating in the presence of short-term wave-induced oscillations (Horrer 1968). The ducted impeller is bidirectional, and the oscillatory components of the horizontal current velocity are recorded but can be separated from the record.

Davis-Wellar propeller type meters are also designed to operate in the presence of waves. These meters utilize two orthogonal propeller assemblies designed to respond linearly to the current vector components so that averaging or filtering of oscillatory movements can be done properly (Wald 1979). Electronic circuits resolve the signals from the propeller sensors into N-S and E-W velocity components, and electronically integrate them over a specified sampling interval. This gives the vector averaged-current over the interval. Oscillatory movements associated with wave action are removed by electronic filters in the averaging procedure.

Drag inclinometer type meters consist of a cylinder with stabilizing fins which is suspended from a pivot point at one end. The drag and lift forces due to the current velocity deflect the meter at a vertical angle which is measured by an inclinometer. The horizontal direction is measured by a compass. Although this type of meter is claimed to measure currents in the wave zone accurately, it may suffer some limitations under these conditions since its response (tilt angle versus current speed) is not linear. This nonlinearity makes it difficult to separate the oscillatory velocity components from the mean current (Daubin et al., 1977). Also, vertical wave-inducted motions will deflect the meter vertically, and movement of the mooring line or significant turbulence in the flow may result in measurement errors.

Electromagnetic current meters measure the instantaneous x- and y-velocity components at a flow sensor which contains a wire coil and two orthogonal pairs of electrodes. The coil produces a magnetic field, and the electrodes measure the voltage gradient across the coil which is induced by the water as it flows through the field. The orthogonal electrode pairs measure the x- and y-velocity components. Since the voltage gradient is proportional to the current speed and there are no moving parts in the sensor, the meter is capable of a fast, linear, highly sensitive component response (McCullough 1977). These characteristics make the meter suitable for use in the presence of waves, since the oscillatory components can be separated either from the records or filtered out by electronically integrating the electrical signals over a specified sampling interval.

Acoustic current meters determine current velocities by measuring the relative travel times of two simultaneous acoustic signals transmitted across the flow sensor. The transmitted acoustic beams are focused on a reflecting plate which returns the signals to the receiving transducers. An acoustic phase shift detection scheme correlates the travel times with the current velocity component along the beam path. Two pairs of orthogonal transducers measure both the x- and y-velocity components. Voltages proportional to the velocity components are resolved electronically using the output signals from the transducers and compass. Since acoustic flow sensors have a fast, linear, highly sensitive response (McCullough 1977), they are suitable for use in the presence of waves. The wave-induced components may be separated either from the records or filtered out by electronically integrating the records over a short sampling interval.

DROGUES AND DRIFTERS

Use of Drogues

Drogues are used to trace the path of moving water near the surface or at fixed depths below the surface. The drogues are released at a given station and are subsequently tracked by recording their positions at short time intervals. The sequence of positions and travel times between positions gives information on the actual path a particle may travel in the currents, and the mean velocities between points along the path. However, the actual paths traced by two drogues simultaneously released at a given point will rarely be the same due to the random components of the velocity field. Thus, several drogues should be released together.

Drogue studies can be used to determine the current patterns in the vicinity of an outfall and to evaluate the movement of a waste plume which either surfaces or remains submerged at a given depth. If a sufficient number of drogues are released at appropriate depths and are monitored at frequent intervals, information can be obtained on the net transport and horizontal dispersion of a waste field, and on the variations in the current velocities along the path followed by the plume. If the drogues are followed long enough, they may indicate where the plume will reach the shoreline, the length of time involved, and the path followed between the discharge point and the shoreline.

Types of Drogues

Most drogues can be classified into one of the following four categories: 1) parachute drogues, 2) cruciform drogues, 3) window shade drogues, and 4) cylindrical drogues. Of these types, parachute and cruciform drogues are the most widely used. Parachute drogues consist of a passenger parachute or smaller pilot parachute which is usually attached to a weighted vertical pipe. The parachute is supposed to remain open and oriented horizontally with its opening facing the currents. However, since some parachutes are denser than water, they tend to hang downward in a closed position at low current speeds. This problem can be reduced by using a spreader bar or ring to help keep the parachute open, and by adding buoyance to the parachute so that it becomes neutrally buoyant. Cruciform drogues usually consist of two or sometimes three identical slotted sheets of plywood, masonite, plastic, or metal arranged in a bi-planar (or tri-planar) crossed vane. The vanes can also be constructed of canvas or cloth stretched across a solid frame. Window shade drogues consist of a rectangular sheet of plastic film, canvas, or cloth suspended from a spreader bar and bridle at the top, with a weighted spreader bar at the bottom. The rectangular drogue should hang approximately vertical with its plane surface oriented perpendicular to the direction of the horizontal flow. However, this orientation, which maximizes the drag forces of the currents, may not always be achieved under actual field conditions. Cylindrical drogues include various vertically oriented cylindrical objects which have been used to trace currents (e.g., 55 gallon drums, drift poles, wooden barrels).

Most drogues are really drogue-buoy systems consisting of a small marker buoy which is tracked at the surface and a larger submerged drogue portion with is set at the desired depth by a connecting line between the two. The drogue portion must be weighted and ballasted so that the drogue assembly has sufficent negative buoyancy to keep both the drogue and connecting line in their intended vertical orientation, and to keep the buoy mast upright. The connecting line must remain close to vertical so the drogue will be measuring currents at the desired depth.

Drogues are intended to passively drift with the currents at a specified depth. In reality, some errors are introduced in the drogue trajectories by wind drag on the exposed portion of the marker buoy, by the relative surface current drag on the submerged portion of the surface buoy, and, for deep drogues with long lines, by the relative current drag on the connecting line. Since surface currents are generally faster than the deeper currents, it is important to design drogues so that the projected area of the submerged drogue is maximized and the projected area of the surface buoy is minimized. Large drogues are much more difficult and cumbersome to launch and retrieve from a boat than smaller designs, especially when many drogues are involved. However, since the accuracy of the measurements generally increases with the size of the submerged drogue, it is best to use the largest size practicable in a given situation. The surface buoy should be the minimum size required to keep the system buoyant and trackable at the surface. The wind forces on the exposed portion can be minimized by using a buoy which is almost completely submerged and which has only a thin radio antenna or small radar transponder protruding upward for tracking. Buoys with considerable freeboard or with larger tracking devices such as flags, radar reflectors, or flashing lights may be subject to significant external wind forces. If possible, it is best to avoid performing drogue studies under high wind conditions, especially when trying to measure the lower current speeds in deeper waters.

Uses of Surface Drifters

Surface drifters are used to measure the average path of currents at the surface. Drift bottles and drift cards are the types most commonly used. Vertical drift cards and drift bottles are useful for evaluating the movement of effluents which reach the surface. Horizontal drift cards, which float horizontally on the surface, may be used to determine the potential movement of surface slicks due to oils or other floatables which form a surface film. Because these movements are influenced largely by the wind, horizontal drift cards should be released under several different meteorologic conditions.

Surface drifters provide a rough estimate of the travel times between the release and recovery allowing a net drift rate to be computed. No information is given on the actual flow path or the velocity variations along the path. However, they do provide information as to whether or not a surface waste field can be expected to reach the shoreline, and, if so, where it will make contact and approximately how long it may take.

Types of Surface Drifters

Drift bottles are long-necked glass bottles partially filled with sand ballast so that only 0.25 to 1 in of the bottle neck remains above the surface. The bottle size is typically 4 to 6 ozs (Grace 1978). Each bottle contains a readily visible postcard with instructions requesting the return of the card with information on the location and time of recovery and generally the offering of a reward.

Drift cards are available in several different forms. Most common types are either drift envelopes, which are plastic envelopes containing instructions on a return card, or plastic drift cards, which are rectangular in shape with identification numbers and instructions stamped into them. As with drift bottles, the information requested is the location and time of recovery and a reward is generally offered for their return. Horizontal drift cards are designed to float horizontally and, therefore, measure transport in the surface film or upper millimeter of the water column. As a result, they are strongly influenced by the wind and do not really measure the surface currents. Vertical drift cards are designed so that only one edge remains at the surface, with the card retaining a vertical orientation in the water column. This is accomplished either by using a negatively buoyant card with a foam flotation strip on the upper edge or a positively buoyant card with a weight strip on the lower edge. Both vertical drift cards and drift bottles measure the average horizontal transport in the upper few feet of the water column, and thus provide a measure of the surface currents. A good design will attempt to minimize the effects of wind on the drifter by minimizing the ratio of the sail area (area exposed to the wind) to drogue area (submerged area exposed to current). Drift bottles may be better in this respect since the exposed bottle necks are much narrower than the submerged part of the bottles.

Since the recovery rate of surface drifters is generally low, many drifters must be released at each station in order to obtain an adequate amount of information. The drifters are usually deployed by boat, but may also be released by airplane. Because of their compact size, it is easier to release a large quantity of drift cards than drift bottles. A good drifter must be durable enough to survive at sea, reach the shore through the surf, and should attract attention once it reaches the shore.

Use of Seabed Drifters

Seabed drifters measure the average path of currents near the sea floor. They are useful for determining the fate of waste materials subject to transport by bottom currents. This includes settleable solids and any portion of the effluent which remains near the bottom. The drifters provide information on the net movement of a waste field along the bottom, including where the waste field may reach the shoreline, and a rough estimate of how long it may take. If a sufficient number of drifters are recovered, they may indicate areas of possible shoreline contamination.

The success of a bottom drifter study depends on reasonable recovery of the drifters. The drifters generally have labels attached which request the location and time of recovery and promise a reward. The condition of the drifter should also be recorded since, if the rod becomes detached, the saucer will float and therefore measure the surface currents rather than the bottom currents. The drifters may be recovered either offshore by commercial fishermen using bottom trawls or bottom gill nets, or more often at the shoreline. The recovery rate will depend on recreational access to the beaches, the intensity with which the beaches are used, and on how extensive the commercial fisheries are in the area. Recovery rates from less than 5 percent to over 50 percent have been reported. Therefore, many more drifters should be released than are expected to be recovered in order to obtain a sufficient amount of data. The information obtained from drifters recovered offshore by commercial fishermen is probably more accurate than data from shore-recovered drifters, since in the latter case no information is provided on the time of first contact with the shore or possible movement in the surf zone. In either case, the only information provided is the point of release, the point of recovery, and a rough estimate of the travel time between the two points.

Types of Seabed Drifters

Woodhead type drifters are generally used. These devices resemble umbrellas in shape, consisting of small plastic dished saucers with a long thin plastic rod attached at the center. Typical dimensions are 18 cm (7 in) for the diameter of the saucer and a 0.65 cm (0.25 in) diameter rod about 54 cm (21 in) long. The rod terminates in a sharpened point and a small, weighted collar [about 6 g (0.2 oz)] is attached near the end (Grace 1978). The saucer has a slight positive buoyancy so it tends to hover slightly above the sea floor and drift with the bottom currents; the weight collar causes the pointed end of the rod to lightly drag along the bottom.

Seabed drifters may be deployed either at the surface by a boat or low-flying small plane, or they may be taken to the bottom and released by divers. Several drifters may be released at the same location on the bottom, even if deployed at the surface, by attaching them to a salt spool which dissolves after the drifters reach the bottom. However, because the rate of descent of the drifters is fairly slow, the actual release point on the sea floor will differ from the known release point at the surface. This difference increases with increasing depth and subsurface current velocities.

DYE STUDIES

Dye studies, using fluorescent tracers, can be very useful in determining the behavior of waste plumes, as well as indicating general circulation patterns in the vicinity of discharge sites. A suitable tracer can be injected either directly into the waste stream before it discharges, or into the receiving water at some point near the discharge site. The injection may be a single dose or a continuous release. The movement of the waste plume and the horizontal and vertical dispersion of the effluent can be determined by measuring the concentration distribution of the dye tracer both temporally and spatially after the initial injection. The dilution rates and the spatial and temporal distribution of contaminants at a given distance from the discharge site can then be evaluated. Three-dimensional distributions can be obtained by sampling at various depths.

SPECIFICATIONS FOR FIELD WORK

After selection of the appropriate method(s) for obtaining the necessary oceanographic information, specifications are needed for the general types of equipment to be used, number of measuring devices, location of measurement stations (drogue/drifter release points or dye injection points), and frequency and times of measurements. If a dye study is to be done, type of dye, appropriate concentration, measurement technique, and frequency should be specified. This section discusses specifications for current meters, drogues and drifters, and dye studies.

Current Meters

The type of fixed current meter selected depends on the importance of wave motion at the site and available equipment. The wave motion expected at the site should be evaluated to determine if data obtained could be affected by wave-induced orbital motion. If so, a non-Savonius type of curent meter should be used. Calibration and visual inspection should be made of the current meters before and after use to detect other sources of error such as fouling of the rotor or the sensor probes. In-situ current meters set for at least 5 days at a time are preferred to current meters deployed for short periods from a boat. The current meter array should include meters set near the surface, at mid-depth, and 1.5 m (5 ft) above the bottom. The exact depths are determined after reviewing the available current data to locate depths where different currents exist, the expected height of rise of the plume, and the current measurement objectives (e.g., whether movement of the waste field or sediment movement is of primary interest).

Current meter location decisions also depend on the current measurement objectives. One meter array should be located near the discharge site. As a minimum, currents should be measured continuously or at least for 15 to 30 min during all 5-day spring and neap tide periods, on a quarterly basis, when receiving water samples are collected.

Drogues and Drifters

The resolution of information obtained during a drogue or drifter study depends on the number of drogues or drifters followed, the frequency with which positions are fixed (recorded), and the accuracy of the method used to fix positions. The use of drogues is recommended over drifters because drogues give information on the flow path and current speeds along the path and also because of the low recoverability of drifters and poor estimates of travel times obtained from drifters. However, these factors have to be considered along with the cost of tracking the drogues.

If certain types of drogues are more suitable for the wind and current conditions at the site, then those types should be recommended in the monitoring program. The drogues should be released over the diffuser at the approximate level to which the plume rises during the time of year of the study. At least five to six drogues should be released each time. The drogues' positions should be traced at half-hour intervals up to a distance of 3.7 km (2 nmi) from the outfall or for a total of 10 hr. Successive vector plots showing drogue position over time should be provided on large-scale nautical charts.

Drifters could be used if the primary concern was whether a surfacing waste plume might reach shore or if only an approximate travel time were needed. The choice of drifter type would depend on deployment method, cost, and distance from shore. Vertical drift cards are the easiest to use and are more durable than glass bottles. The drifters should be released at the point of discharge or the middle of the diffuser.

Dye Studies

Specifications for a fluorescent dye study to be done as part of a given monitoring program include the time of year for the dye study, the type of dye to be used, the approximate concentration to be achieved in the receiving water, the length of time of dye injection and measurement, and suggestions on the measurement technique to be used. Each of these aspects will be discussed briefly.

The purpose of a dye study is to determine the horizontal and vertical extent of the plume and the direction of the plume's movement. A dye study should be performed at a time of minimum stratification, which occurs most often in the fall or winter, and at a time of maximum stratification, which occurs most often in the summer. Density profiles collected during water quality monitoring surveys should be checked to identify or verify periods of maximum and minimum stratification. The selection of the type of dye depends on the degree of adsorption which could occur at the site, the cost, and availability of chemicals. The dye tracers most commonly used at the present time are rhodamine B and rhodamine WT. Rhodamine B has fairly strong adsorptive tendencies, and may be adsorbed onto suspended solids, sediments, plankton, aquatic plants, and sampling and injection equipment. Rhodamine WT is the preferred choice because it is much less susceptible to adsorption than rhodamine B, although it is about twice as expensive. Both dyes are available in liquid form, which is strongly recommended over powdered forms due to ease of handling. The solutions should be adjusted to the same density as sewage effluent, if necessary, by mixing with methanol or by addition of salt.

The effects of temperature, degradation, and photodecomposition must also be considered. The fluorescence of a sample will vary with temperature, although this can be adjusted easily with a temperature correction curve. Chemical degradation can be a problem in the presence of strong oxidizing agents such as chlorine. The degradation rate is usually low at the chlorine levels typically encountered in the field, but it should be evaluated for dye injected directly into a chlorinated waste stream. Photodecomposition rate can be estimated from a control solution of dye which is exposed to sunlight on the boat and monitored for the duration of the study.

Concentrated dye solutions must be diluted to appropriate levels before being released. Because the fluorometer calibration curve reverses direction for dye concentrations greater than about 1.0 ppm (Turner Designs 1976), dye studies should be designed so that the dye concentrations measured in the field are below this level. Dilution techniques can be used for higher levels where discrete water samples are collected. However, this is not done easily when an on-ship flow-through apparatus is used or when in-situ measurements are taken with a towed submersible fluorometer. The dye should be added to the effluent just before it enters the outfall for a period of about 6 hr, begining 3 hr before the current reverses. The concentration in the effluent stream should be 0.2 ppm above the background fluorescence of the seawater. The background fluorescence of moderately polluted water may be as high as 100 to 200 parts per trillion, and raw sewage has a background fluorescence of about one part per billion (Turner Designs 1976). The pigments present in blue-green algae can yield substantial background fluorescence if large concentrations are present. Although appropriate optical filters can be used to minimize this problem, background fluorescence should always be determined from seawater samples before releasing the dye.

The dye can be measured using a submersible fluorometer or a continuous pump and shipboard fluorometer. Submersible fluorometers, if used, should be towed at a depth approximately equal to the equilibrium level of the plume. Measurements should be made for about 12 hr beginning 3 hr before the start of the dye injection. When dye studies are conducted, turbidity, temperature, and salinity profiles should be taken at 3-hr intervals throughout the tidal cycle at stations near the outfall. These data will help in locating the plume and in interpreting dye study results. More detailed discussions of dye studies and fluorometers are included in Feuerstein and Selleck (1963), Smart and Laidlaw (1977), Turner Associates (1971), and Wilson (1968).

Navigation-Position Determination

Position determination is important for siting and returning to sampling stations and for tracking drogues. Although several different methods are available, the most commonly used methods for nearshore coastal surveys are Loran C, electronic range-positioning systems with onshore transponders, horizontal sextant readings from aboard ship, and theodolite readings from shoreline stations.

Loran C is the government sponsored radio navigation system selected for use in the coastal zone by the Department of Transportation. The Loran C system has recently been implemented on the West Coast and in Alaska. The previously existing chains on the East Coast, Gulf Coast, and the Hawaiian Islands have been expanded and modernized to provide a complete network of

Loran C staions for the U.S. coastal zone. Receivers automatically resolve Loran C signals and display position with claimed accuracies as high as ± 15 m (± 50 ft).

Portable electronic range-positioning systems are available from several manufacturers and have claimed accuracies as high as ± 1 to 3 m (± 3 to 10 ft). These systems are the preferred method for position determinaton. Electronic range-positioning systems are limited to line-of-sight measurements. The maximum range varies with the manufacturer. However, most types have ranges sufficient for nearshore applications. It should be noted that at very short ranges (<50 yd) electronic range-positioning system errors may be disproportionately large.

Visual methods of position determination such as sextant or theodolite readings are limited to use under conditions of adequate visibility. In the sextant method, the position of the observer on-board a boat is fixed by measuring two horizontal sextant angles between three charted objects, with one object being common to both angular measurements. In the theodolite method, the position of an object in the water (for example a drogue, or a boat at a sampling station) is determined by simultaneously taking two theodolite readings from separate shoreline stations. By knowing the locations of the theodolite stations and the horizontal angles to the observed object, the position is fixed. The accuracy of positions determined with a sextant or theodolite varies with the precision of the instruments, the experience of the operators, the horizontal distances involved, the magnitudes of the angles, the strength of the three-point fix (for sextant readings), the scale of the charts, and the accuracy with which the positions are plotted.

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U.S. GOVERNMENT PRINTING OFFICE : 1982 O-393-769/278